SCIENTIFIC VALIDATION OF ANTI-CANCER, ANTI-TUMOUR AND ANTI-OXIDANT ACTIVITIES OF SIDDHA HERBO- MINERAL FORMULATION "BHRAMASTHIRAM" IN VARIOUS CELL LINE STUDIES.

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GOVT. SIDDHA MEDICAL COLLEGE,

CHENNAI-106

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **Scientific Validation of Anti-cancer, Anti tumour and Anti-oxidant activities of Siddha Herbo-mineral Formulation "*Bhramasthiram*" in Various cell lines studies** is a Bonafide and genuine research work carried out by me under the guidance of **Dr.M.D.Saravanadevi M.D(S).,**Post Graduate Department of Gunapadam, Govt. Siddha Medical College, Arumbakkam, Chennai-106 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **Scientific Validation of Anti-Cancer, Anti-tumour, and Anti-Oxidant Activities of Siddha Herbo-mineral Formulation "Bhramasthiram" in Various cell line studies** is submitted to the Tamilnadu Dr.M.G.R.Medical University in partial fulfillment of the requirements for the award of degree of M.D (Siddha) is the Bonafide and genuine research work done by **Dr.S.SEMALATHA** Under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

Date: **Seal and Signature of the Guide**

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ABBREVIATIONS

ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AST	Aspartate Amino Transferase
ANOVA	Analysis of Variation
BA	Bhramasthiram
BUN	Blood Urea Nitrogen
CT	Computed Tomography
COX	Cyclooxygenase
CMC	Carboxy Methyl Cellulose
CAMP	Cyclic Adenosine Monophosphate
CACCS	Calcium Activated Chloride Channels
CLCA2	Calcium activated chloride channel regulator2
CFTR	Chloride Channel Activator
CPCSEA	Committee for the Purpose of Control and Supervision of Experimental Animals.
DMEM	Dulbecco's Modified Eagle's Medium
DNA	DeoxyRibo Nucleic acid
DC	Differential Count

DSC	Differential Scanning Calorimeter
EDX	Energy Dispersive X-ray Spectrometry
FDG-PET	F-18 Fluoro-2-deoxy-D-glucose
FAD-Assay	Flavine Adenine Dinucleotide
FTIR	Fourier Transform Infrared Spectrometry
GOT	Glutamate Oxaloacetate Transaminase
GPT	Glutamate Pyruvate Transaminase
HPV	Human Papilloma Virus
HDL	High Density Lipoprotien
ICPMS	Inductively Coupled Plasma Mass Spectrometry
IAEC	Institutional Animal Ethical Committee
ICMR	Indian Council of Medical Research
LDL	Low Density Lipoprotein
LD50	Lethal Dose
MCV	Mean Corpuscular Volume
MRI	Magnetic Resonance Imaging
MTT	3-(4, 5-Dimethylthiazol-2-yl)-2, 5- Diphenyl Tetrazolium Bromide
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
NCRP	National Cancer Registry Programme
NCCS	National center for cell line

OECD	Organisation for Economic Corporation and Development
OSCC	Oral squamous cell carcinoma
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
PET	Positron Emission Tomography
RBC	Red Blood Cells
SEM	Scanning Electron Microscope
SEM	Standard Error Mean
SGOT	Serum Glutamate Oxaloacetate
SGPT	Serum Glutamate Pyruvic Transaminase
VLDL	Very Low density Lipoprotein
WDS	Wavelength Dispersive Spectroscopy
WBC	White Blood Corpuscles
WHO	World Health Organization
XRD	X-Ray Diffraction

1. INTRODUCTION

அரிது அரிது மானிடராய் பிறப்பது அரிது

-அவ்வையார்

Human being is the most wonderful creature in the world. To born a human is said to be a boon. Humans, in all the aspects such as intelligence, cognitive faculties etc., finds no equal with other species. With his extra ordinary sixth sense he enjoys all the wonders and wealth of nature. He always has the choice to alter the source of life by making changes which may enhance his life and existence. At times the choices one makes in life can make his life a bane. Sometimes certain things are imposed upon him of which he has no control. One such uncontrollable fate is the disease which might be nonfatal or fatal such as Cancer, AIDS, and Tuberculosis.

"Medicine is a science of uncertainty and the art of probability"

- William Osler.

"It is more important to know what sort of person has a disease than to know what sort of disease a person has"

-Hippocrates.

Cancer is a major life threatening disease. It is also said to be a lifestyle disorder which is knowingly or unknowingly incorporated in our day to day activities. Lifestyle modifications due to the nature of the job, social environment, lack of physical activity, alcohol, smoking, stress, plastic products usage, junk food, culture, sleep disturbances cause severe impact in health and cause cancer.

Cancer is believed to be derived from the Greek word, *"KARNIKOS"* which means *crab or crayfish*. "It stick to the part stuberner". It comes from the appearance of the cut surface of a solid malignant tumor [1]. Researchers defined cancer as a disease characterized by uncontrolled or unregulated proliferation of cells with the potential to invade or spread to other parts through blood and lymphatic vessels. [2]

Cancer is the second leading cause of mortality. In India, Tumour is the third cause of mortality. There are more than 100 types of cancers. In no particular order, following are some types of cancer prevailing commonly among people:

- Lung Cancer
- Oral Cancer
- Cervical Cancer
- Breast Cancer
- Colorectal Cancer
- Uterine Cancer etc...

Lung and oral cancers are affected mainly by undesirable habits such as alcohol, smoking and tobacco consumption. Smokeless tobacco such as chewing of betel and tobacco with snuff are most important risk factors. Cervical cancer starts in cervix and the lower narrow part of the uterus. Mostly, cervical cancers are triggered by the Human Papilloma Virus (HPV, especially HPV-16). Other factors including radiation, lack of oral hygiene, nutritional deficiencies, preserved or salted food are involved in the oral cancers [3]. Many women are affected by breast cancer because of irregular sleeping habits, late or increased maternity age, etc. Stomach cancer and colorectal cancer are caused mostly by the intake of unhygienic junk food and ulceration that is resulted from regular spicy foods. Such kinds of food habits are unhealthy.

This dissertation deals with the significance of Oral and Cervical cancers and the ways to cure these dreadful diseases by means of Siddha Medicine.

In this category, malignant tumours of the cervix ranks first in the female mortality rate. It is the fourth most common cancer in women with an estimated 528,000 new cases in 2012. As per IARC Globocan 2012 there were an estimated 266,000 deaths worldwide in 2012, which accounts for about 7.5% of all female cancer deaths. Almost nine out of ten (87%) cervical cancer deaths occur in the less developed regions. In India, nearly 122,844 women diagnosed with cervical cancer and out of this 67,477 women die annually from this disease.[4]

Cervical cancer remains to be the second most common cancer in women between the age group of 15-45 years.[5] Sexually transmitted Human Papilloma Virus (HPV) infection is the most important risk factor for cervical intraepithelial neoplasm and invasive cervical cancer. [6]

HPV serotypes 16 and 18 account for nearly 76.7% of cervical cancer in India. HPV has been found to be a necessary but not sufficient cause for cervical cancer, of the more than 100 HPV types, 18 have been categorized as high-risk types, while the rest are low-risk types for cervical cancer [7].

HPV prevalence among cervical cancer patients in India has varied from 87.8% to 96.67%. Molecular studies have shown that HPV-16 and 18 are the two most common high oncogenic types found in invasive cervical cancer and among these, HPV-16 have been found more common. The Pap smear test is intended to detect the cells that can develop in the cervix of the uterus as a result of HPV.

OSCC remains the major cause of mortality and morbidity in patients with head and neck cancers. There are about 14.1 million of new cases estimated and 8.2 million people die out of cancer every year, and 32.6 million people are living with cancer in 2012 worldwide [8]

Oral cancer is the most common cancer in India, as 4 in 10 of all cancers are oral cancers. Nearly 130,000 people succumb to oral cancer in a year which is approximately 14 deaths per hour. [9]

In the last decades there were great advances in the diagnosis of cancer as well as in the fields of molecular oncology. However, the cure rate of most cancers remains low. In Modern system of medicine, the main strategies of treating patients with cancer are surgery, radiation and chemotherapy. Some of the most commonly used drugs for oral cancer are Cisplatin, 5-fluorouracil (5-fu) and other drugs such as carboplatin, Bleomycin, Methotrexate are also used. Prolonged usage of the drugs produces many side effects.[10] The current use of chemotherapy is accompanied with difficult side effects. It inhibits bone marrow stem cells proliferation leading to immune suppression. Radiotherapy, which is widely used in the world, is also accompanied by a great deal of side effects. Lymphocytes are most readily affected by radiation resulting in prolonged T-cell suppression. Other side effects such as, Alopecia, bone necrosis, lung fibrosis, skin devascularization, ulceration, nausea, vomiting, and renal damage are also associated with all types of conventional therapies.

Cisplatin which is used to treat different types of cancer such as cancers of head and neck, cervical cancer, on small lung cancer produces certain ill effects like renal

damage, hearing loss especially, with high pitched sound, easy bruising, occasionally amenorrhea in female, loss of fertility, tinnitus, numbness or tingling sensation of fingers.

Whereas 5-fluorououracil produces diarrhoea, redness and peeling on the palm of the hand and sole of the feet and occasionally blue tinged nails, sensivity of skin to sunlight, sore throat and blurred vision. [11]

Bleomycin produces allergic reactions, fainting, confusion, breathing difficulties.

Methotrexate produces birth defects, weakness, diarrhoea, and occasionally skin rashes, breathlessness, darkening or lightening of the skin, blurred vision, and loss of fertility.

Mephalan is very effective multiple myeloma, used in ovarian cancer, produce bone marrow depression, infections, diarrhoea, arthralgia. mucositis, thrombocytopenia. Cytarbine produces leukopenia, thrombocytopenia, anemia, mucositis, thrombocytopenia bone marrow, Mylo suppression, Nausea, vomiting.

Siddha medical system delivers huge like of treatments for different kinds of life threatening disease including cancer. Cancer is explained in the name of *Putru* (undermined growth) which gives the direct meaning and as *Arputhapun* (spectacular tumour) and *Vanmeegam* (pre-cancerous growth. Siddha physician consider some types of cancer growth with the symptoms of V*ippuruthi*(multifaceted growth their practice,*Kazhalai,Pilavai* etc. [12]. In ancient Siddha literature, oral cancer referred as "*Kanna Putru*" or "*Vaai Putru*" and cervical cancer referred as "*Yoni putru"*.[13]

"*Nature itself is the best physician"*

-Hippocrates.

"Each and every plant, a magnificent creation of nature, Has magnetical power of healing.

-Bible

Siddha Medical Science is one of the ancient Indian traditional medicine followed by Tamil speaking peoples worldwide. Unlike other medicine systems, siddha

system hubs not only the prevention and cure of a disease but also emphasizes in *'kaya kalpa'* which is a way of making one's body immortal. The word "*kaya"* means body and "*kalpa"* means stone and the word "*kayakalpa"* means sturdy as a rock and ageless.

The term *Siddhi* refers to a yogi state. Siddhars are said to be the yogis, having lived a complete life. [14] Siddhar are well known practitioners in preparation of a herbo-mineral formulations through a wide range of processing such as purification using herbal juice thereby reducing the toxicity of the metals.

"Natural forces within us are the true healers of disease".

-Hippocrates

The treatment aspects in Siddha system insists the usage of herbs at first followed by inorganic preparations such as paspam and chendurams as mentioned in the following lines.

வேர்பாரு தழைபாரு மிஞ்சினக்கால்
மெல்ல மெல்ல பற்ப செந்தூர பாரே"

\- புதார்த்தகுண சிந்தாமணி [15]

Pathangam is referred as sublimation. The process of sublimation by which solid substance such as camphor, Sulphur, Sub chloride of mercury, Corrosive sublimate, benzoin stc., are brought into the state of vapour by heat and condensed again into a solid by cold without melting them.

The product of sublimation is enriched with Nanoparticles which aids in targeted drug delivery and site specific action. Nano medicine is the application of knowledge of nanotechnology in science and medical procedures. It is a field of applied science devoted to the control and manipulation of matter on a scale smaller than one micrometer. Nano medicine are used globally to improve the treatment and lives of patients suffering from range of disorders, like cancers, auto immune disorders, degenerative disease, multiple sclerosis, asthma and emphysema.

Nano medicine therefore can play an important role in ensuring enough of the drug enters the body and stays in the body for long periods and is targeted specifically

the parts that need treatment. Nano medicine has high bio availability, longer shelf life period, targeted action with minimal side effects.

There are a wide variety of Nano medicines in siddha system which is being successfully used in routine clinical practice such as *Velli parmam. Linga chendooram, kshya kulandhaga chenduram, Mupoora chendooram ,Thalaga parpam, Naga parpam e*tc.[17].

Bhramasthiram is one such medicine enriched with Nano particles. Bhramasthiram is a herbo mineral formulation consisting of four drugs, which is specified as an effective anti-cancer drug mentioned in siddha literature "**The Pharmacopeia of Siddha Research Medicine"**.

Bhramasthiram is most powerful weapon accordingly in Hindu Mythology. It was said that when Bhramasthiram was discharged there was neither a counter attack nor a defense that could stop it. Over the coming years, the benefits of Nano medicine and new diagnostic tools will be felt by an increasing number of patients with considerable impact on global health.

Nanoparticles have the potential to overcome certain limitations in conventional chemotherapy, like lack of solubility selectively and the drug resistance. Importance of Nano therapeutic drug delivery system include enhanced bio distribution, increased circulation of time of drug, prolonged half-life, increased intracellular concentration of the drug and many more. Eg Velli parpam dosage one in six part of tip of crow beak.[18]

- நந்தி மை - கன்னப்புற்று,யோனிப்புற்று
- கௌரி சிந்தாமணி செந்தூரம் - புற்று[19]
- குரு பதங்கம் - கன்னப்புற்று,
- ஸ்வர்ண புஷ்ப ரச செந்தூரம் - யோனிப்புற்று
- பஞ்ச பாஷாண செந்தூரம் - கன்னப்புற்று
- கௌசிகர் குழம்பு - கன்னப்புற்று, மார்புப் புற்று
- சித்திர மூலக் குளிகை - கன்னப்புற்று,இலிங்கபுற்று
- இரச பற்பம் - யோனிப்புற்று
- புற்று பதங்கம் - கன்னப்புற்று
- நமச்சிவாய செந்தூரம் - யோனிப்புற்று

- அஷ்ட பைரவ செந்தூரம் - கன்னப்புற்று
- கந்த ரச வில்லை - யோனிப்புற்று

An integrated approach is the need of the day to manage cancer using the growing knowledge gained through scientific development. The emerging integrative Nano medicine model of cancer treatment recognizes the importance of herbo mineral medicine which there drug literally evident as told by great scientists Siddhars. Therefore, the author is interested in validating anti-cancer and anti tumour, anti-oxidant activities of "*Bhramasthiram*" through standardization, physico chemical, bio-chemical analysis in in-vitro cell line studies.

2. AIM AND OBJECTIVES

AIM:

The aim of this study is to establish the scientific validation of the Anti-cancer, Anti-tumor and Anti-oxidant activities of ***BHRAMASTHIRAM*** for Cancer. In the present medical world, there is a need for proper treatment for Cancer. The aim of this study is evaluation of a new drug for the management of cancer.

OBJECTIVES:

The main objective of the present study is to highlight the efficacy of ***BHRAMASTHIRAM*** on ***Cancer***, the following methodology was adopted to evaluate the drugs and its standardization studies.

- Collection of various Siddha and modern literature relevant to the study.
- Identification of drugs in this formulation.
- Preparation of ***BHRAMASTHIRAM*** as per the Siddha classical text.
- Physicochemical and phytochemical investigation of the test drug.
- Evaluate bio-chemical analysis of the test drug to derive acidic and basic radicals.
- To estimate the present of elements, functional groups and particle size through instrumental analysis of the trial drug.
- Evaluation of the Acute and 28 days repeated oral Toxicity of the test drug according to OECD guidelines.
- Evaluation of the pharmacological study of the drug through the following activities
    - Evaluation of Anti-Cancer activity
    - Evaluation of Anti-tumor activity
    - Evaluation of Anti-oxidant activity of ***Bhramasthiram***

3.REVIEW OF LITERATURE

3.1 DRUG REVIEW

3.1.1. GUNAPADAM ASPECT

VEERAM (Savveeram)

- Perchloride of Mercury, Mercuric chloride
- Perchloride of Mercury was first used as a therapeutic agent for venereal diseases during the middle of the eighteenth century in western countries. But for many centuries the Perchloride of Mercury has been used in India for the treatment of various disorders.

Synonyms:

- *Meenachimynthan, Kotchiveeram, Poovindhusevagan, Sarakku chunnam, Parangi Pasanam, Sarathin sathru, Parimithru*
- According to the above version the synonyms are *Virpan, Aathi, Vellai chenduram, Sevagan, Pidaragan, Varagathinthalai*

Origin

Veeram is one of the natural in 64 *padanam*s. Naturally it is obtained in Himalaya hills. Now we use the artificial one. It is called as *Savveeram.* It is identified in seventh century and used for venereal diseases during the middle of the eighteenth century in western countries.

Synthetic preparations (*Vaippu)*

Ingredients:

- Calomel - 80 parts
- Culinary salt - 80 parts
- Copper sulphate - 40 parts
- Alum - 20 parts
- Potassium nitrate - 20 parts
- Fuller's earth - 20 parts
- Sulphate of iron - 10 parts
- Ammonii chloridum - 5 parts

These ingredients are taken and triturated well and placed in a bottle its mouth is closed and sealed with a mud pasted cloth and burnt. After cooling, the per chloride of mercury is found to be deposited over the lid as a thick layer.

Specialities

- It is a very important drug for preparing *Parpam*, *Chendooram* and *Guru*
- It is having the capacity to make *kattu* of 64 drugs
- Make *karam into kattu* medicine form.

Methods of purification and detoxification:

Perchloride of mercury is as such quite toxic and it should be used only after purification and detoxification. Perchloride of mercury 35gm is consolidified with pepper decoction (Piper nigurm) for 6 hours. Then it is buried within the pepper poultice. Sodium chloride 650 gm and camphor 35 gm are mixed well and kept in a mud pot in which the above poultice is buried and burnt for some hours with low intensity fire to get the purified form of perchloride of mercury.

Method I

Camphor is mixed with tender coconut water and placed in a mud pot. Perchloride of mercury is tied in a cloth and soaked in the pot without touching the water and the pot is burnt for half an hour.

Other Method of Purification

Method II

Alum 35 gm and Camphor-35 gm. are powdered well and mixed together. Perchloride of mercury 35 gm is taken single piece and consolidified by the above mixture gradually. Perchloride of mercury is carefully watched not to become fume.

Properties and actions of *Veeram*

Characters

Taste	:	Bitter and salty
Potency	:	Hot
Pirivu	:	Pungent

It has got body improving tonic and antiseptic and ulcerogenic properties.

Panchabootha Amsam: *Theyu*

"அப்பனே"! தேயுதான் வீர மாகும்"

According the *Veeram* is *Appubootham* in "*pasanathil Panchapootham*"

In *Pasanapirivil Veeram* considered as *a Theyu* (fire) *Bootham.*

- *Veeram* is classified as a T*heyu sarakku* in *panchabootha sarakku* and also *the*
- *Karasarakku, and peesasarakku*.

The following five have been specifically mentioned as *Pancha boodha paadaanam* in the text "*Pacchai Vettu 16*".

1. Earth	-	*Prithivi*	:	*Arithaaram*
2. Water	-	*Appu*	:	***Savveeram***
3. Fire	-	*Theyu*	:	*Gowri*
4. Air	-	*Vayu*	:	*Vellai*
5. Sky	-	*Vinn*	:	*Lingam*

"அரிதாரம் பிருதிவியே யாகி நிற்கும்
ஆனதொரு சவ்வீர மப்பு வாகும்
பெரிதான கௌரியோ தேயு வாகும்
பின்னுமோர் வெள்ளையோ வாயு வாகும்
அரிதான லிங்கமா காச மாகும்
அருந்தியாயப் பச்சையதா யாட லாகும்"
-குணப்பாடம் தாதுவகுப்பு

However, in *Nandheesar's Kalaignanam the panchaboodha Paadaanas* have been mentioned in different way.

Substances Antagonist to *Veeram* (*Sathru sarakku)*

- Iron (*Irumbu)*
- Magnet *(Kantham,)*
- Tablesalt *(kalluppu,)*
- Potassium nitrate (*Vediuppu),*
- Zinc *(Thuththanagam)*
- Egg white *(Muttai vellai karu*)

Substance Synergetic to *Veeram*: Copper (*Thurusu*)

Actions

- Alternative *(Udalthetri)*
- Antibiotic *(Kiruminasini*)
- Anti-septic (*Azhugal agatri*)
- Caustic *(Punnundakki*)

General properties (*Pothu gunam*)

"பாடினேன் பிறக்குமுப் பத்தி ரண்டு
பாங்கான தாதுவுடைப் பெயரைக் கேளு
வீடினேன் வீரமொடு வைக்கிரனந் தந்தான் "
"தாதுதான் தந்திரமாய் சித்தர் வைத்த
சாங்கமாம் பேரையினிச் சாற்றக் கேளு
கொங்கியென்சவ் வீரங்கோழித் தலைக்கெந்தி"
-போகர் 7000
"குன்மமொடு குட்டம் கொடியவனி லத்திரட்டு
துன்மாங் கிசப்பெருக்குஞ் சூலைநோய் -வன்மையுறு
காமியப்புண் ணாதியநோய் கணடாற்சவ்
வீரனெனுஞ் சாமிநா மத்தையுச் சரி"

Savveeram is used to cure the following disease Gastric ulcer, leprosy, severe vadha diseases and morbid growth of flesh, throbbing pain associated diseases, venereal diseases, bubo in the groins occurs to the female and male due to forcefulness of sexually contact as explained in the above Tamil verse. This is also for various types of eye diseases.

Dose

- 2 - 4 mg
- Dose above 4 mg will be toxic.

Method of administration of *Veera Jayaneer:*

- The perchloride of mercury and Ammonii chloridum (650 mg each) are taken and dissolved in 500 ml of purified water and administered up to 30 drops.

MAHAVEERA MEZHUGU

Dose: Equivalent to the size of 1-2 pulse grain

Indication

Vatha diseases and venereal diseases.

Dietary restriction Milk and rice.

***VEERA MAATHIRAI (THIRI THODA MATHIRAI*)**

- If given with suitable adjuvants the diseases like fever caused due to the derangement of three *gunmams* will be cured.
- Instead of *milagu decction, Nochi Surasam* (Vitex negundo) is also used by which running nose and shivering fevers are cured.

***SAVVEERA CHENDOORAM* (Hydrargyrm perchloride)**

- If given in sufficient quantity it cures, fever, delirium, severe *vatha* diseases, cholera, *soolai etc.*

VEERA RASA PARPAM

Dosage: 488 mg or required minimum quantity with jaggery.

VEERA NEER (For External application)

- It is used as a disinfectant and for cleaning the ulcers.

VEERA KALIMPU

- 4.2 gram of *Veeram* is ground with 17.5 gm of butter and the paste is used to cure urticaria and to apply on the ulcers.

***VEERA KALIMPU (AMIRTHA VENNAI-AMIRTHA MEZHUGU*)**

- It can be applied on die ulcers like Cancer or Carbuncle of chest
- Clean the ulcers using the water boiled with tamarind leaves.
- It is also used as local application on all swellings, boils etc.

As the *Amirtha mezhugu* contains more quantity of butter than *Veera Kalimbu* it cures the inflammation.

JAYAVEERA RANA SINGI KAYIRU

Adjuvant: Pure water

Indications

- Ulcers, ringworm, pimples, syphilis, scabies, carbuncles, cancer of penis, piles, scrofula, venereal ulcer, anorectal syphilis and different kinds of eye diseases.

OTHER PREPARATIONS

1. *Veera sanjeevi mathirai*

Dose : *Ulunthu alavu* (65gms)

Indication : *Sanny, Suram.*

2.Maha koda surimathirai

Dose : 65gms

Indications : V*ettai, sanny, visa noi*

3. Veeraparpam patchai vettu

Dose : 50mgs- 3days- 6 times

Indications : *Pun,vellai,kiranthi,katti,soolai* .

4. Ramabana chenduram

Dose : Arisi edai

Indications : *Vayu,Veekam,Suram, Megam.*

5. *Thanvanthri sandamarutha chenduram*

Dose : *Pana edai* (480 mg)

Adjuvant : Honey

Indications : *Sunny13, kuttam, Mahotharam, Peeligai, Gunman,* Vaginal cancer.

6. *Vadha marutha melugu*

Dose : *Milagalavu*

Adjuvant : Jaggery

Indications : *Mudakku ,Vadham ,Soolai,Pandu,* Epilepsy,*Parisavayu.*

7. *Chandamarutha chenduram*

Dose : Panavedei (480 mgs)

Adjuvant : Honey.

Indication : 13 *Sunny, Peeli, vadham,* Fistula, *perumpadu*

8. *Veera paspam*

Dose : *Panavedei*

Adjuvant : Honey

Indications : *Sunny, Thosam, kabakatti,vayu.*

Uses of *Veeram* [20]

1. *Veera jayaneer* - Dose 30 drops

2. *Mahaveera mazhugu* - Dose 65 - 130 mgs

3. *Veera mathirai (Thurthada mathirai*)

Dose : *Milagalavu*

Adjuvant : *Notchi* decoction

Indications : Running nose, *kulir suram*,Fever

Saveera chendooram

Dose : 50-100 mgs

Indication : *Suram, sanny, pithavanthi, pedigai, soolai*

Gurupathangam

Dose : 1/2 *Arisiedai* (30mgs)

Adjuvant : Palm jaggery, Dry ginger paste

Indications : *Mehvayu*, various types of ulcers, check cancer, *soolai noi*

Navalogamelughu

Dose : 1/2 -1 *kundri* (65-130mgs)

Adjuvant : Palmjaggery, Dry ginger, Butter

Indications : Fever, *Chunny, Mehanoi, Giranthi pun*

Pattu karuppu

Dose : 2-4 *Arisiedai* (130-260mgs)

Indications : *Suram, mehasuram*, oedema, epilogsy

External use of *Veeram*

Veera neer

Veeram : *65mg*

Water : 240 ltr

- ***Veera kalimbu*** - Apply on the ulcers
- ***Pilavaikku pasai*** - Apply on the carbuncles
- ***Veera pugai*** - 3 days and 6 times

  Indication - *Giranthipum,Araiyapu* non curable ulcers

- ***Veera pugai*** - Cures the oozing ulcers
- ***Veera powder*** - Cures the chronic ulcers
- ***Venkarapodi*** - *18 kuttam, thimir*, snakebite, ulcers, piles, syphilis

- ***Manjal karaseelai*** - Piles, marpani, *neerabeelai, Thimir, karanai katti*

Punpuraikku pugai

Dose : 4.1 gms

Smoke curves *soolai* and ulcers

Moolam pavathiram punnukku pugai

Dose : 4.1gms

smokes cure piles, fistula, ulcers.

Tholaiyu mennar sirodhararo - *Kustarogam, soolai, kaikal mudakku, pun, Vadha karappan*

Akkinipilasthiri - Curves all types of ulcers.

Other preparation

- *Vanga chunam, Thalaga parpam*
- *Ghanthaga thailam, Ayaveera chendhooram*
- *Vaanmezhugu, Thirumoorthi pathangam*
- *Megavirana kalimbu, chandamaarutham*

Signs and Symptoms

The toxic symptoms of mercury chloride are Taste of verdigris, Ulcerative stomatitis, Ulcerative gastritis, Vomiting, Watery diarrhea, Puffiness of face, Fissures on the skin with serous fluid oozing, Morbid thirst, Hiccough, Syncope, Ptyalism, Ulcerative laryngitis Dysphagia, Ulcers in the stomach, Dysentery, Pharyngitis, Throbbing pain in the hypochondral region, Unconsciousness, Convulsions, Death.

Antidotes

1. 20 ml of *Tribulus terrestris* juice should be given to the victim in the morning and evening daily.
2. Paste of the *Indigofera tinctoria* root bark in the dose of a size of *Solanum pubescens,* dissolved in 80 ml of lukewarm water should be consumed twice daily. Decoction of the above drug is also given as an antidote for the poison.

3. 20 ml of juice of *Vemonia cinerea* consumed twice daily which also acts as an antidote.

4. This poison can be neutralized by drinking coconut toddy. Since the period of treatment is not mentioned, all the above prescriptions can be continued till the toxic effects disappear.

5. White yolk of the egg (unboiled) mixed with water or milk should be given often.

6. Tender coconut water also neutralizes the toxicity of Mercuric chloride [20].

3.1.2 MODERN ASPECT OF HYDRAGYRUM PERCHLORIDE

Properties

Mercury(II)chloride or mercuric chloride is the chemical compound with the formula Hgcl2.

This white crystalline solid is a laboratory reagent and molecular compound**.**

PHYSICAL PROPERTIES [21]

Molecular formula	:	Hgcl2
Molar mass	:	271.52g/mol
Appearance	:	white solid
Melting point	:	277ºc,549k,529F
Boiling point	:	304ºc,577K,579F
Density	:	5.44g/cm2
Vapour pressure	:	1.3mm Hg
Refractive index	:	1.859
Storage temperature	:	Store at room temperature
Solubility	:	H2O
Form	*:*	*Powder*
Water solubility	:	7.4g/100ml
Solubility	:	Soluble in alcohol, ether, acetone, ethyl acetate, slighty soluble in benzone, CS_2
Acidity(Pk_2)	:	3.2

Figure1 . Hydragyrum perchloride

Stability	:	Stable, but moisture sensitive and light sensitive Decomposes in sunlight

STRUCTURE

Crystal structure	:	Orthogonal
Coordination geometry	:	Linear
Molecular shape	:	Linear

Mercuric chloride is not a salt but a linear triatomic molecule, hence its tendency to sublime. In the crystal, each mercury atom is bonded to two close chloride ligands.

Preparation

Mercuric chloride is obtained by the action of Chlorine on Mercury by addition of Hydrochloric acid to a hot, concentrated solution of Mercury (I) compounds such as the nitrate.

$$HgNO3+2HCl\ HgCl2+H2O+NO2$$

Heating a mixture of solid Mercury(II) Sulphate and Sodium Chloride also affords volatile HgCl2, which sublimes and condenses in the form of small rhombic crystals.

APPLICATIONS

- Mercuric chloride is a catalyst for the conversion of acetylene to vinyl chloride, the precursor to polyvinylchloride.
- It is used as a depolarizer in batteries
- It is being used in plant tissue culture for surface sterilization of explants such as leaf or stem nodes.
- Mercuric chloride is occasionally used to form an amalgam with metals mercury(II) chloride was used as a photographic intensifier.
- Syphilis was frequently treated with mercuric chloride before the advent of antibiotics.it was inhaled ingested injected and applied topically.

3.1.1. SIDDHA ASPECT OF *KARIYUPPU* (SODIUM CHLORIDE):

Synonyms:

Sotruppu, Kadaluppu, Veettuppu, Ilavanam, Samudra lavanam.

Chemical name:

Sodium chloride

Actions:

- ❖ Stomachic
- ❖ Laxative
- ❖ Emetic
- ❖ Anthelmintic, Febrifuge

General properties (*Pothu gunam):*

"அளத்திலுறை நல்லுப் பனல்வாதம் மாற்றுங்
குளத்துநோய் தன்னைக் களையுங்- கிளைத்தகப
ஆசுடைய வல்லைநோய் அஷ்டகுன்ம மும்போக்குங்
காசினியுள் மாதே கழறு"

- பதார்த்த குணசிந்தாமணி

Pitha vatham, lymph adenitis, tumour, kapham, liver disorders, 8 types of gastric ulcer, indigestion, distended abdomen, vayu and retention of urine will be cured. Appetite will be increased.

Purification

- ❖ Common salt-1 part, vinegar(or)pure water-7 part, common salt is dissolved in pure water or vinegar filtered. The filterate is boiled till it reaches semi consistency, then little amount of lemon juice or butter milk is added to it and isolated. the same process is reapeted for 10 times to get the purified.
- ❖ It is also purified by plantain stem juice, sea water and rain water.

Other preparations

- **Uppu parpam**

  Dose : ¼ th size of a dhal for 32 days

  Adjuvent : Honey or warm water.

Indication : Throbbing, Head ache, Mental disorder, Menorrhagia, ascites with pain and syncope due to excess kapha.

- **Uppu chenduram**

  Dose : 65 mg to 130 mg, with suitable adjuvant.

  Indication : Indigestion, Gastric ulcer.

- **Kariyuppu Thiraavagam**

  Dose : 5 drops

  Adjuvent : water

  Indication : Dyspepsia, Indigestion, Vadha disease, weakness.

Medicinal uses

- ❖ The salt is placed and heated at the pricked sites of the pile and thorn, the toxicity will be cured.
- ❖ Equal ratio of salt and tamarind are dissolved in water and boiled and the mixture is taken in semi-solid consistency and applied over the swelling and sprain by which hematoma will subside.
- ❖ It is also used as one of the ingredients in tooth powders.
- ❖ Insects entered in the ear, generative organs, anus will come out on spraying the salt water.
- ❖ Dissolved salt water is given through the rectum to remove the worms in the large intestine.

3.1.2. MODERN ASPECT OF SODIUM CHLORIDE

Vernacular names

Sanskrit name : *Lavana, Samudra Lavana, Dronilavana*

English name : Common salt, table salt, muriate of sodium, muriate of soda.

Arab : Milhus, Aajin.

Pers	:	Namake-khurdam
Hindi name	:	Namak, Lun Nun
Duk	:	Nimak
Bengali name	:	Nimok,
Gujarati name	:	Lesu
Telugu name	:	Mithun
Tamil name	:	Uppu,
Malayam name	:	Uppu

Figure: 2 Sodium chloride

Sodium chloride is obtained when the sea water evaporates by isolation. Common salt is cultivated in the eastern coast of Tamil nadu, Cheyyur, Choonam bedu, Marakkanam, Athira pattinam, Arumuga neri and Tuticorin.

Physical characters

Colour - White, pale and ash colour.
Odour - No characteristic odour
Taste - Salty in taste
Solubility - Soluble in water, in soluble in alcohol

Actions

- Stomachic,
- Laxative
- Emetic, Anthelmintic
- Febrifuge

General properties

It is one of the essential substance of our body. It Is excreted from the body by sweat, tears and urine. Deficiency may lead to many diseases like anemia, G.I tract infections, excessive thirst and skin diseases. The sea salt contains a little amount of iodine and prevents goitre. When sodium chloride is taken in small amounts, stimulates appetite and causes thirst. When a concentrated solution is applied over the cut wounds,

it produces irritation and inflammation. It also controls the secretion of the respiratory tract. Sodium chloride is mainly excreted through the urine.

Medicinal uses

- The salt is triturated with water and applied on the site of the poisonous bite to reduce the poison. It is dissolved in water and instilled as drops to minimize the poisonous effect of the poison.
- One spoon table salt is dissolved in 546ml of hot water and gargled to reduce inflammation of throat and gingivitis.
- It is used to give fomentation for swelling and pain. The salt is bundled and dipped in a cloth and dipped in boiling oil applied over the swelling when it is warm.
- An isotonic solution of sodium chloride in sterile water is given as intravenous infusion in case severe diarrhea, with dehydration.
- If the salt is placed and heated at the pricked sites of the pile and thorn, the toxicity will be cured.
- Equal ratio of salt and tamarind are dissolved in water and boiled and the mixture is taken in semi-solid consistency and applied over the swelling and sprain by which hematoma will subside. [23]

GUNAPADAM ASPECT

Puliyaarai (Oxalis corniculata)

Synonyms: *Pulikkeerai,Puliyakkerai.*

Vernacular Names

English	:	The indian sorrel
Malayalam	:	Pulli-yarala
Kannada	:	Pullam-purachi-sappu
Telugu	:	Pulli-chintaku,Pullacinta
Sanskrit	:	Changeri
Hindi	:	Amboti pi-datti

Parts used	:	Leaf
Taste	:	Sour
Character	:	Coolent
Division	:	Sweet

Action

- Stomachic,
- Refrigerant
- Diuretic,
- Astringent

General Character

"பித்த மயக்கமறும் பேருலகின் மானிடர்க்கு
நித்தமருள் வாதகபம் நேருமோ- மெத்தனவே
மூலக் கிராணியறும் மூல வுதிரமறூங்
கோலப் புளியாரை க்கு."

-அகத்தியர் குணவாகடம்

Indications -

Piles, diarrohoea and anti-dote for insect's bite.

Medicinal uses

- The leaf decocation is used to treat fever, tumour, eye disease, skin warts, Molluscum, ulcer, Reduce the toxicity of datura. [24]
- The plant rubbed down with water, boiled and the juice of white onions added, the mixture is applied to the head in bilious headaches.
- The fresh juice of leaf and decoction of jujube fruits and ginger, alkaline water and curdled milk butter make a oil used to treat for prolapse of the rectum. [25]

Oxalis cornuculata

Taxonomical classification

Kingdom	:	Plantae
Clade	:	Angiosperms
Clade	:	Eudicots
Clade	:	Rosids
Order	:	Oxalidales
Family	:	Oxalidaceae
Genus	:	Oxalis
Species	:	*Oxalis cornuculata*

Figure 3 Oxalis cornuculata

Distribution

Throughout India, upto 3000m in all moist exposed localities.

Description:

A diffuse annual or perennial procumbent or more or less erect creeping acid herb,6-25 cm in height, entire, cuneate at the base.

Stems

Stems are prostrate to sub erect to 40cm long, slender, turnched, often rooting at nodes, covered with spreading flexible hair,

Leaves	:	leaves with three leaflet, alternate, sometimes appearing almost whorled on short lateral stems, green or purple
Petiole	:	05-1.7cm long, with spreading flexible hairs
Stipules	:	Usually 2-3mm long, broad with free truncate apex, nearly glabrous to densely covered in long hairs
Flowers	:	yellow, axillary or sub umbellate
Inflorescence	:	1-5 flower
Fruits	:	Loculicidal cylindrical capsules
Seed	:	Numerous, dark brown, transversely striate
Parts used	:	Whole plant

Chemical constituents

Different parts of the plant especially root contain various compounds such as β-sitosterol, botulin,4-hydroxybenzoic acid, ethylgallate, methoxyflavones, apigenin and 7-o-β-D glucopyranoside, flavonoids, tannins, phytosterols, phenol, glycoside, fatty acids, galacto-glycerolipid and volatile oil. The leaves contain flavonoids, isovitexine and vitexine-2"-o-Beta-D-glucopyrunoside. malic acid from stem, tartaric acid citric acid from stem, leaves. It is rich source of essential fatty acids like palmitic acid, oleic, linoleic, linoleic and stearic acids [26]

Macroscopy

Type	:	Trifoliate
Colour	:	Light green
Shape	:	Obcordate
Margin	:	Entire
Apex	:	Emarginatge
Base	:	Symmentrical
Odour	:	Characteristic
Taste	:	Pleasantly sour taste
Arrangement	:	Alternate
Size	:	Width about 1.4cm to 1.6cm
Length	:	0.8cm to 0.9cm
Surface	:	Upper-Smooth dark green
Lower	:	Smooth pale green
Texure	:	Hairy [27,28]]

Microscopy

The leaf is thin with less prominent midrib. The lamina is uniform in thickness and the lateral veins do no project beneath the surface of the lamina [29]

Powder microscopy

Presence of epidermal trichomes, calcium oxalate crystals and stomata**.**

Nutrional Composition [30]

The leaves have been found to be rich in moisture, total carbohydrate, crude protein, crude lipid hence it can be alternative vegetable during emergency. The leaves of *Oxalis corniculata* Linn. exhibit rich in mineral contents like

Table 1.Nutritional parameters

Nutritional parameter	**Quantity**
Moisture	82.42+-0.5
Carbohydrates	24.67+-0.5
Crude proteins	22.28+-0.5
Crude lipids	23.75+-0.5
Sodium	1.12+0.02%
Potassium	2.17+0.31%
Calcium	2.510.08%
Nitrogen	3.5610.70%
Magnesium	0.25+0.03%

these mineral components are vital in regulating various metabolic pathways in human body [31].

Properties and uses

The plant is sour, astringent, thermogenic, cooling , anodyne ,anti-inflammatory, digestive, carminative, liver tonic, diuretic, febrifuge, antibacterial, vermifuge, astringent, dysmenorrhea, styptic, hepatitis antiseptic, anthelmintic, dysentery, and burning sensation, It is also useful in dyspepsia, haemorrhoides, anemia, tympanitis, fever, dysentery, diarrhea, scurvy, corns, warts, excrescence of the skin, inflamed ulcers, cephalalgia, oththalmopathy, toxicity, cardiopathy, haemorrhages,, amenorrhea, strangury, hepatopathy and burning sensation.

Hypoglycemic, antihypertensive, chronotropic effect, uterine relaxant, anti-psychotic, CNS stimulant, ant yeast, inotropic effect, smooth muscle relaxant. The plant is traditionally used as remedy for convulsions in children and for healing fractured bones (Mohamd and Mir, 2000; Kirtikar and Basu, 1975; Ameenahet al., 1993)

- The plant has an acid taste, due to the presence of acid oxalate of potassium to which is due its property in domestic use of removing ink-spots or iron-stains from linen.

- The fresh leaves juice is believed to relieve the intoxication of datura it is also believed, on application to remove fibers over the cornea or opacities if the cornea.
- The plant is rubbed down with water, boiled and the juice of white onions added the mixture is applied to the head in bilious headaches.

Vellai Saranai(Trianthema decandra)

Synonyms : *Vellai saranai, vellaisaradai, sakthisattaranai, virichakam*

Vernacular Names

English	:	Spreading hog weeds
Malayalam	:	Tavizhama
Telugu	:	Tella-ghhaijeru
Sanskrit	:	Punarnava
Duk	:	Bees-khupra
Parts used	:	Leaf,Root
Taste	:	Bitter
Character	:	Heat
Division	:	Pungent, Diuretic
Action	:	Expectorent, Laxative

Gerneral character

"வித்திரிதி மூலம் விழிப்படலம் மார்பு
தத்து சுவாசங் தனிக்கருப்பை - யுற்ற
கருச்சிதறச் செய்சூசி காவா த மும்போம்
விருச்சிகத்தின் பேரை விரி."

-அகத்தியர் குணவாகடம்

Indications

Various uterine diseases, *Kapha* diseases, Tumours, Piles, Asthma, Swelling, Pterigium.

Medicinal uses

- The root is used to treat with scabies, Ulcers, Sinusitis, Tinea infections, Leucorrhea, Scrotal swelling.

- ❖ The root juice mixed with *Tabernaemontana divaricate* juice treat with eye diseases.
- ❖ The leaf decoction is used to treat with eczema.

Trianthema decandra

Taxonomical classification

Kingdom	:	Plantae
Subkingdom	:	Tracheobionata
Super Division	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Caryophyllidae
Order	:	Caryophyllales
Family	:	*Aizoaceae*
Genus	:	*Trianthema*
Species	:	*decandra*

Figure 4. ***Trianthema decandra***

Distribution

Distributed in tropical and subtropical regions.

Description

Structurally the plant is glabrous, branched, and prostrate weed with branches, glabrous with a firm tap root up to 2mm long.

Stem	:	The stems are fleshy, prostrate, and often reddish in colour and usually are slightly angular, pubescent and branched.
Leaves	:	Green in colour, succulent, oval, opposite, unequal in size
Stem	:	Herbaceous, sparsely branched, procumbent angular

Petiole	:	0.5 to 3 cm long, ovate to oblong shaped, green color
Flowers	:	Small 5-lobed, pink, purple or white in colour
Fruits	:	Represents cicumscissile capsules, partly exerted from the persistant perinathand contain 2-8 seeds.
Seeds	:	kidney shaped, approximately 2mm in diameter, hairless, are brownish to black in colour.
Root	:	Cylindrical, gradually tapering measuring up to 8cm long. [33]

Chemical constituents

The presence of carbohydrates, glycosides, flavonoids, alkaloids, steroids, Saponins and alkaloid like punarnavine, Trianthemol, 15hydxoxymethyl-2,6,10,18,22,26, heptamethyl1-14-methlene-17, trianthemine, sitosterol, stigma sterol, triaconitene neolignan, leptorumal, and ecdysteiods.

Properties and uses

Trianthema decandra is used in India for treatment of variety of ailments. It is used as a single drug in form of dried powder or decoction for jaundice, liver diseases, anemia, cough, headaches, epilepsy, odema, asthma etc. As the whole plant possess diuretic indicated in anasarca, cystitis in case of dribbling of urine, in dropsy, edema and ascites.

- The roots are used for **Cathartic, epilepsy.**
- The leaf juice is dropped in each nostril in headache, migraine, abdominal gas.
- The decoction of leaves is given for regularized the cycle. rheumatism, edema
- The root paste is applied for ulcers, itching, poor eyesight, night blindness.

Macroscopic strucuture

Aerial part	:	pale green colour
Odour	:	Characteristic strong
Taste	:	Bitter& disagreeable [35]

Table 2. Nutrional parameter [34]

Nutritional Parameter	Quantity(mg/g±SD
Moisture	80.0± 2.2
Ash	348.0±6.2
Total lipid	20.0c±0.6
Saponifiable lipid	11.2±0.8
Non saponifiable lipid	8.8a±0.2
Total protein	91.9±3.2
Fiber	430.0±8.2
Carbohydrate	30.d±1.2
Energy (kcal/100 g)	76.01
Vitamin A	0.81±0.2
Vitamin b2	2.02±0.3
Sodium	44.00±1.4
Potassium	51.60±5.2
Zinc	0.20±0.02
Copper	0.02±0.1
Iron	6.44±0.4
Manganese	0.04±0.01
Nickel	0.03±0.006

Antaratamarai (Pistia stratiotes)

Synonyms : *Aagayathamarai*

Vernacular Names

English : The water –lettuce (Tropical Duckweed)

Malayalam : Kodda-pail,Akasa-tarma

Kannada : Anthar-gange

Telugu : Anthar-tamarai,Agasa-tarma

Sanskrit : Kumbhika,Vari-parni

Hindi : Jal-kutbhi

Parts used : Leaf, root

Taste	:	Bitter
Character	:	Heat
Division	:	Pungent
Action	:	Refrigerant, Demulcent Laxative, Emollient

General character

"அழுகிரந்தி குட்டம் அடர்ந்த கரப்பான்
புழுவுறுமக் கூடுமுதற் போகும்-அழகாரும்
இந்திரநீ லக்கருங்கண் ஏந்திழையே! எப்போதும்
அந்தரக் தாமரையா லாய்."

-அகத்தியர் குணவாகடம்

Indication

It cures leprosy, eczema, hemorrhoids, dysentery, cough and various inflammation.

Medicinal uses

- Leaf paste is externally applied for pile, prolapse and inflammation.
- Leaf boiled with vinegar, squeezed out the water, and then the extract is banded it cures leprosy, eczema, leaves with coconut milk and rice used for dysentery.

Pistia stratiotes

Taxonomical classification

Kingdom	:	Plantae
Clade	:	Angiosperms
Clade	:	Monocots
Order	:	Alismatoles
Family	:	Araceae
Subfamily	:	Aroideae
Tribe	:	Pistoia
Genus	:	Pistia
Species	:	stratiotes

Figure 5 Pistia stratiotes

Distribution : Distributed in tropical and subtropical regions.

Description

P. stratiotes is a free-floating, stoloniferous plant with sessile leaves in rosettes. Leaves pale-green, up to 20 cm long and 10 cm wide, mostly spathulate to broadly obovate with a rounded to truncate apex, with 7-15 prominent veins radiating fanwise from the base of both surfaces, in particular the lower surface, covered by a dense mat of white woolly hairs.

Inflorescence

Axillary, solitary, ascending; spathe 1.3-1.5 cm long, convolute and adnate to the spadix below, spreading above, whitish; spadix with a single pistillate flower at base, and with 2-8 staminate flowers flowers are above, shorter than the spathe. Flowers unisexual, the perianth wanting; stamens 2; ovary 1-locular, with numerous ovules, the style slender, the stigma penicillate.

Fruit : Thin-walled,

Seed : cylindrical, rugulose

Part used : Whole plant

Chemical constituents

Stigmasta-4,22-dien-3-one, stigmasterol, stigmasteryl stearate, and palmitic acids. Plant gave 2-di-C-glcosylflavones of vicenin and lucenin type, anthocyanin-cynidin-3-glucoside, luteolin-7-glycoside and Mono-C-glcosyl flavones – vitexin and orientin.

- The High level of acid detergent fibre, cellulose, hemicellulose and lignin present.

Medicinal uses [37]

- The Plant is bitter, pungent flavored having cooling, laxative property.

  It is useful in "Tridosha," fever, and diseases of blood.

- The root is laxative, emollient, and diuretic. Leaves infusions have been mentioned in the folklore to be used for dropsy, bladder complaints, kidney

afflictions, hematuria, dysentery, and anemia, plant is considered antiseptic, ant tubercular, antitussive, demulcent, the plant is used as an anodyne for eyewash.

- The ash of plant is applied to the ringworm of the scalp. Leaves are used in eczema, leprosy, ulcers, piles, and syphilis.
- The ash mixed with rose water and sugar they are given for cough and asthma.
- Juice of leaves boiled in coconut oil is applied externally in chronic skin diseases.

Table 3.Nutritional parameters[36]

Nutritional Parameter	Quantity
Crude protein	8.62%
Total Nitrogen	1.32
Ether extract	1.16%
N-free extract	46.62%
Ash	21.12%
Crude fiber	19.13%
Potassium	5.56
Calcium	3.24%
Magnesium	1%
Phosphorus	0.26%
Sodium	0.61%
Iron	0.26%
Copper	42ppm
Zinc	181ppm

PALM JAGGERY [38]

- Palm jaggary is almost like a jaggery that is made out of sugarcane juice.
- Palm jaggery is made from the extract of palm tree in southern India.
- It has an intense, earthy taste or reminiscent of chocolates in taste.
- The palm jaggery obtained after processing is darker and richer in colour.

- It is slight salty to taste but much healthier of the two. Due to its cooling effects over human body, it is of high value. These trees are also known as toddy palm tree or palmyra tree.

Synonyms

Tamilnadu	:	Karupatti
Karnataka	:	Thaati bella
Mangalore	:	olebella

Figure 6 Palmyra tree

- Jaggery is a rich source of many vital minerals that are required by the body for normal growth and functioning. Jaggery is used since ancient times to treat problems such as dry cough, common cold and asthma. It is beneficial in treating conditions like indigestion and constipation.
- Jaggery helps in promoting relaxation of muscles, nerves and blood vessels, thus enhancing their functions.
- Jaggery is a rich source of iron and is therefore, very good for anemic people, as it increases the hemoglobin level in the blood.
- Jaggery has strong antioxidant properties and protects our body cells from the damage caused by free radicals.
- Jaggery has the ability to purify blood and helps in regulating the liver function. A mixture of jaggery and dry ginger powder taken with warm water can stop hiccups.
- Jaggery may be used in the creation of alcoholic beverages such as palm wine.
- Jaggery is used in natural dying of fabric and in hookahs in rural areas of Pakistan and India.
- Jaggery helps in regulating blood pressure and also helps the body to get rid of all the toxins.
- Jaggery has potassium content that may help in reducing bloating and water retention.
- It cleanses the respiratory tract, intestines, food pipe, lungs, and stomach. It also helps to wipe out the toxins from the body, leaving you healthy and fit.
- Relieves Constipation, reduce the weight. Helps to migraine.

Table 4. Nutritional parameter of palm jaggary

Nutrition parameter	**Quantity**
Protein	0.35%
Fat	0.17%
Minerals	0.74%
Carbohydrates	90.60%
Calcium	0.06%
Phosphorus	0.06%
Iron	2.5%(mg/gm)
Nicotinic acid	5.24(mg/100 gm)
Vitamin B1	24.0(mg/100gm)
Riboflavin	432.0(mg/100gm)
Vitamin C	11.0(Mg/100gm)

Figure 7 Palm Jaggary

3.2. DISEASE REVIEW

3.2.1. Siddha Aspect of the Disease

Hippocrates (ca. 460 BC – ca. 370 BC) described several kinds of Cancer, referring to them with the Greek word *Karkinos* (Crab or Crayfish). This name comes from the appearance of the cut surface of a solid malignant tumour, with "the veins stretched on all sides as the animal the crab has its feet, whence it derives its name" [39].

In Siddha system of medicine, Cancer is referred to *Vippuruthi* or *Putru*. ***Putru*** which gives the direct meaning and as *Arpudham* and *Vanmeegam*. Generally, a chronic tumour or swelling or ulcer is first identification of *Putru* in *Siddha* system.

Tumours grow gradually and finally look like a *Putru* or cauliflower.For the pupose of diagnose and treatment following reference books evaluates great ideas about Cancer.

"புற்று நோயை மௌனப்பகைவன்
மறைந்திருந்து கொல்லும் பகைவன்"
-திருமந்திரம்

1. *YugiVaidhiyaChinthamani*
2. *AnubogaVaidhiyaNavaneetham*
3. *Pulipani 500*[40]
4. *Agathiya Vaidhiya Vallathi*
5. *Agathiyavaithiyavallathi 60*[41]
6. *Anubogavaidhiyabhramaragasiyam*
7. *Agathiyavaithyagandam*

great *Siddhar* Agatthiya in his *Vaidhiya Vallathi 600* had explained cancer and its different categories.

"போக்குமே திமிர்வாத மண்டைசூலை புற்றுடனே
கேட்குமே அரையாப்பு பவுத்திரத்தைக் கேள்
தண்டு சூலையொடு லிங்கப் புற்றே"
-அகத்தியர் வைத்திய வல்லாதி

"நாமப்பா கருங்கிரந்தி யோனிப்புற்று
ஆமப்பா கருவழிக்குங் கிரந்தி லிங்கப்புற்று"
-யூகி வைத்திய சிந்தாமணி

"இருபுடரி நுனி நாசி சிலந்தி புற்று
தடர்சிலந்தி படர்சிலந்தி அல்குல் புற்று
பின்கரப்பான் முங்கரப்பான் அண்டப் புற்று
துணிவாத உந்திப்புண் துடையில் புற்று
கீழ்நாக்கு மேல்நாக்குப் புற்றுப் போமே"
-புலிப்பாணி 500

The unique saint Pulipani also dealt with different type of cancer in his *Pulipani 500.*

"ஓமேனி குழிப்புற்று யோனிப்புற்று
ஒளிவான இடிப்புற்று கன்னப்புற்று"

Theriyar vaitihyam1001

"விறையிறேன் முக்கரத்தின் கரப்பான் புண்கள் இடிப்புற்று குறிப்புற்று யீரல்புற்று
விறையுறேன் யுடிகரப்பான் குறவுகுஷ்டம் வீச்சுடனே புறவீச்சு விஷ நீர்ப்புற்று
பையப்பா புற்றுவகை யெல்லாங் கட்டும் பயக்குமப் பாரசபற்பம் பரிந்து பாரே.

-அகஸ்தியர் கண்ம காண்டம்

In this medical system of life, the cancerous growth and tumors are headed as *Arputha viranangal* and *Arputha kattigal*.According to *Yugimamunivar Vaithiya Chinthamani* 800 part I, some kinds of cancer clarified under different systemic diseases.

Yugi classifications of diseases are compared with Modern system of medicine by names of symptoms for quick and easy approach [(42)].

For example,

- *Ukkarasoolai* is understand as prostatic cancer
- *Vilperuvayiru* can be compared with Testicular cancer
- *Mamisamagotharam* and *Kalperuvayiru* as cancerous growth within the abdomen. Cancer is considered as *Vippuruthi.*

Causes

- Taking excessive amount of salt and pungent.
- Taking large quantity of fish and meat.
- Making sleep in day time.

Types of *Vippuruthi*:

Vippuruthi is classified into seven types,

Curable type of *Vippuruthi:*

1. *Karpa Vippuruthi*
2. *Kuvalai Vippuruthi*
3. *Pitha Vippuruthi*
4. *Oodu Vippuruthi*

Incurable type of *Vippuruthi:*

1. *Santhu Vippuruthi*
2. *Sethuma Vippuruthi*
3. *Vatha Vippuruthi* [43].

Karppa Vippuruthi:

- Gastric regurgitation
- Pain in side and lower abdomen
- Dryness of skin
- Lower abdominal swelling like pregnancy
- Head ache

Kuvalai Vippuruthi:

- Pain in lower back, anal region and side of the chest
- Fever with shivering
- Cough with expectoration
- Abdominal pain and swelling

Vatha Vippuruthi:

- Pain and swelling in the lower abdomen, this swelling looks like a frog
- Fever, Wound in the abdomen
- Pus discharge from the wound and abdominal distension

Pitha Vippuruthi:

- Hematemesis
- Paleness of the skin
- Burning sensation all over the body
- Shivering
- Mental disorder
- Hiccup
- Tastelessness of the tongue
- Fever
- Dehydration
- Hematoma and abdominal pain

Sethuma ***Vippuruthi****:*

Small tumour and abscess in the abdomen, abdominal pain, fever, cough and swelling of the body.

Santhu Vippuruthi:

Swelling in the side of the abdomen, this swelling is characterized by

- Shining
- Hardness
- Cool and itching

Oodu Vippuruthi:

- Fever
- Blackish discolouration of skin
- Abdominal pain
- Giddiness
- Vomiting
- Diarrhoea and body pain.

The great *Siddhar* Agatthiya in his *Vaidhiya Vallathi 600* had explained cancer and its different categories.

For example,

- *Ukkarasoolai* is understand as prostatic cancer
- *Vilperuvayiru* can be compared with Testicular cancer
- *Mamisamagotharam* and *Kalperuvayiru* as cancerous growth within the abdomen.

Appearance:

Cancerous growth appears like,

- Solid tumor - *Kazhalaikatti*
- Spreading ulcer
- Initially like warts then growth develops as turtle shell with oozing.
- Hyper pigmentation of skin, affects hair follicles and destroys entire body.

Classification:

Cancer classified into 3 types according its spreading nature (Metastasis)

- ❖ Skin and its structures
- ❖ Muscles
- ❖ Blood vessels and bones

Causes

- ➢ Vitamin and mineral deficiency
- ➢ Increased sexual activity
- ➢ Prolonged starvation, Excessive use of tobacco
- ➢ Excessive intake of hot and spicy food
- ➢ Taking excessive amount of salt and pungent food stuffs
- ➢ Taking large quantity of fish and meat
- ➢ Sleeping in the day time.

Yoni Putru

Yoni means birth passage. This is cervix of uterus. So the cancer of cervix is known as Yoniputru. It is also called *Karuppai kazhunthu putru.*

Symptoms

- ➢ Growth in cervix appearing like small grains.
- ➢ Discharge like honey
- ➢ Hardening of surface
- ➢ Profuse bleeding
- ➢ Constipation
- ➢ In some patients discharges with intolerable foul smell.
- ➢ Oliguria and anuria, Administration of diuretics causes haematuria.

Discharges classified into 3 types

1. Viscous yellowish discharge
2. Yellow discharge with mucous
3. Bloody discharge due to non-healing cervical ulcer and cancer of cervix.

The SiddharYugi in his Vaidhiya Sinthamani mentioned the symptoms of *Yoniputru* in different types as follows,

Kuruthiyoni [44a]

″ திறமான வுபத்திரவ மதிகங் காணும்
தெளியாத ரத்தமுடன் சீழ்நீர்ப் பாய்ச்சல்
கறமான நுரையுடனே நோயுண்டாகும்
கடினமாஞ் சதையுடனே குத்தல் காணும்
நிறமான மஞ்சளுடன் கசரோ கந்தான்
நிலையாது வல்குலிலே புழுவோ மெத்த
மலமான சொல்லதுவு முளுத்தாற் போல
மஞ்ஞையா னிறம்போல் மசக்கும் பாரே.″
-யூகி முனி

Profuse bleeding with mucous, micro ulcers like pits on the wall of cervix, discoloration of os.

Kuruthicheezhyoni [45]**:**

″பாரேதாமன் வேதனை மிகவுண்டாகும்
பாங்கான சீழுடன் ரத்தங் காணும்
சீரேதான் ஒழுக்குடன் நாற்றமாகும்
சிதறியே பலபேத வண்ணங் காட்டும்
நேரேதா நிதம்பத்தின் ஸ்தனந் தன்னில்
நெடிதான ரோகத்தை மேவச் செய்யும்
வேரேதான் சொன்னபடி சிகிச்சா சாரம்
விரித்திட்டர் யூகிமுனி விளக்கந் தானே.″
-யூகி முனி

Bleeding with mucous sometimes in multicolor, foul smelling discharge with bad odour, spread to whole uterus.

Mamisamagotharam[44b]**:**

″போக்கான மாமிசந்தான் வளர்ந்து மீறி
பொருமியே அடிவயிற்றில் கல்லைப் போலத்
தாக்கான சடந்தானு முலர்ந்து வற்றி
தவிக்குமே யடிக்கடிதான் கண்ணீர் தேடி
வாக்கான மதுரமொழி குளறிப் பேசி
வாய்வுதா னடிக்கடிக்கு மேலே நோக்கும்
நீங்கான மலசலமிதில் மாமிசங் காணும்
நேரான மாமிச மகோதரத்தி னேரே.″

There are various treatments available especially for *Yoniputru, vaai putru* in Siddha medicine.

3.2.2 Modern Aspects of Cancer

Cancer known medically as malignant neoplasia, is a broad group of diseases involving unregulated cell growth. In Cancer, cells divide and grow uncontrollably, forming malignant tumours, which may invade nearby parts of the body.

The Cancer may also spread to more distant parts of the body through the lymphatic system or bloodstream. Not all tumours are Cancerous; benign tumours do not invade neighbouring tissues and do not spread throughout the body. There are over 200 different known Cancers that affect humans.

History:

Cancer has existed for all of human history. The earliest written record regarding Cancer is from circa 1600 BC in the Egyptian Edwin Smith Papyrus and describes Cancer of the breast. Hippocrates (ca. 460 BC – ca. 370 BC) described several kinds of Cancer, referring to them with the Greek word *Karkinos* (Crab or Crayfish).

This name comes from the appearance of the cut surface of a solid malignant tumour, with "the veins stretched on all sides as the animal the crab has its feet, whence it derives its name." Galen stated that "Cancer of the breast is so called because of the fancied resemblance to a crab given by the lateral prolongations of the tumour and the adjacent distended veins". Celsus (ca. 25 BC – 50 AD) translated *Karkinos* into the Latin Cancer, also meaning crab and recommended surgery as treatment. Galen (2nd century AD) disagreed with the use of surgery and recommended purgatives instead. These recommendations largely stood for 1000 years.

In the 15th, 16th and 17th centuries, it became acceptable for doctors to dissect bodies to discover the cause of death. The German professor Wilhelm Fabry believed that breast Cancer was caused by a milk clot in a mammary duct. The Dutch professor Francois de la Boe Sylvius, a follower of Descartes, believed that all disease was the outcome of chemical processes, and that acidic lymph fluid was the cause of Cancer. His contemporary Nicolaes Tulp believed that Cancer was a poison that slowly spreads, and concluded that it was contagious.

The physician John Hill described tobacco snuff as the cause of nose Cancer in 1761. This was followed by the report in 1775 by British surgeon Percivall Pott that Cancer of the scrotum was a common disease among chimney sweeps. With the

widespread use of the microscope in the 18th century, it was discovered that the 'Cancer poison' spread from the primary tumor through the lymph nodes to other sites ("metastasis"). This view of the disease was first formulated by the English surgeon Campbell De Morgan between 1871 and 1874. [45]

Epidemiology of Cancer:

Nearly seven lakh Indians die of Cancer every year, while over 10 lakhs are newly diagnosed with some form of the disease. According to the latest World Cancer Report from the World Health Organization (WHO), more women in India are being newly diagnosed with Cancer annually. As against 4.77 lakh men, 5.37 lakh women were diagnosed with Cancer in India in 2012.

In terms of Cancer deaths, the mortality rate among men and women in India is almost the same. While 3.56 lakh men died of Cancer in 2012 in India, the corresponding number for women was 3.26 lakh. One in every 10 Indians runs the risk of getting Cancer before 75 years of age.

Cancer of lip and oral cavity has emerged as the deadliest among Indian men while for women, it is breast Cancer. The top five Cancers in men are lip/oral cavity, lung, stomach, colorectal and pharynx, while among women they are breast, cervix, colorectal, ovary and lip/oral cavity.

The global Cancer burden jumped to 14.1 million new cases in 2012, with WHO saying the marked increase in breast Cancers must be addressed. The International Agency for Research on Cancer (IARC) 2012 estimated 14.1 million new Cancer cases and 8.2 million Cancer-related deaths occurred in 2012, compared with 12.7 million and 7.6 million, respectively, in 2008.

The most commonly diagnosed Cancers worldwide were those of the lung (1.8 million, 13% of the total), breast (1.7 million, 11.9%), and colorectum (1.4 million, 9.7%). The most common causes of Cancer death were Cancers of the lung (1.6 million, 19.4% of the total), liver (0.8 million, 9.1%), and stomach (0.7 million, 8.8%).

Projections based on IARC 2012 estimates predict a substantive increase to 19.3 million new Cancer cases per year by 2025, due to growth and ageing of the global population. More than half of all Cancers (56.8%) and Cancer deaths (64.9%) in 2012

occurred in less developed regions of the world, and these proportions will increase further by 2025 [46].

Cervical cancer has emerged as a second most common cause of cancer deaths among Indian women aged between 15 and 44 years, according to a report by Spain-based international public health institution Institute Catalàd' Oncologia (ICO) Information Centre.

On an average, **India** reports about 122,000 new cases of cervical cancer annually, with around 67,500 women succumbing to the disease, accounting for 11.1% of total deaths related to cancer. Cervical cancer comes next to breast cancer in terms of mortality rate in Indian women. The two preventive strategies for cervical cancer include screening and vaccination. The report says just 3.1% women in India get screened, leaving a large population vulnerable to death from the disease.

Causes:

Cancers are primarily an environmental disease with 90–95% of cases attributed to environmental factors and 5–10% due to genetics. Only 5–10% of all cancer cases can be attributed to genetic defects, whereas the remaining 90–95% have their roots in the environment and lifestyle. The lifestyle factors include cigarette smoking, diet (fried foods, red meat), alcohol, sun exposure, environmental pollutants, infections, stress, obesity, and physical inactivity.

The evidence indicates that of all cancer-related deaths, almost 25–30% are due to tobacco, as many as 30–35% are linked to diet, about 15–20% are due to infections, and the remaining percentage are due to other factors like radiation, stress, physical activity, environmental pollutants etc.

It is nearly impossible to prove what caused a Cancer in any individual, because most Cancers have multiple possible causes. For example, if a person who uses tobacco heavily develops lung Cancer, then it was probably caused by the tobacco use, but since everyone has a small chance of developing lung Cancer as a result of air pollution or radiation, then there is a small chance that the Cancer developed because of air pollution or radiation [47].

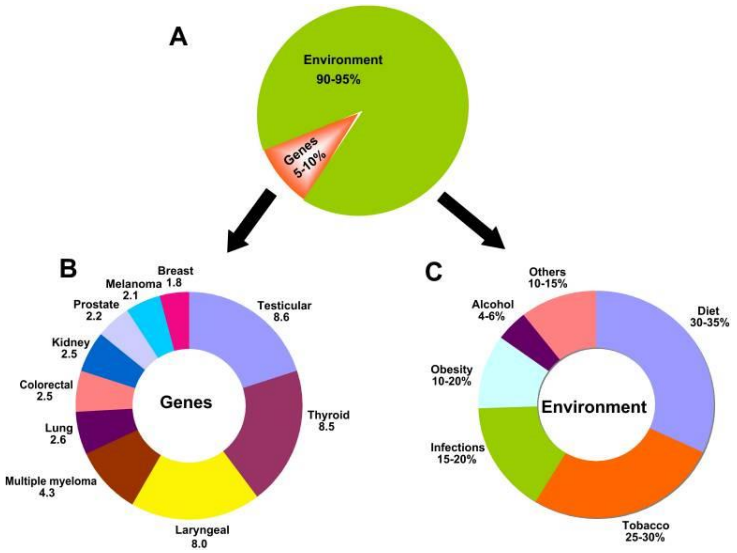


Fig.No 8. Cause of cancer

Other Medical Factors

As we age, there is an increase in the number of possible cancer-causing mutations in our DNA. This makes age an important risk factor for cancer. Several viruses have also been linked to cancer such as,

- Human Papillomavirus (a cause of cervical cancer)
- Hepatitis B and C (causes of liver cancer)
- Epstein-Barr virus (a cause of some childhood cancers)
- Human immunodeficiency virus (HIV)
- Anything else that suppresses or weakens the immune system - inhibits the body's ability to fight infections.
- Increases the chance of developing cancer.

There are **five** broad groups that are used to classify Cancer.

- Carcinomas are characterized by cells that cover internal and external parts of the body such as lung, breast, and colon Cancer.
- Sarcomas are characterized by cells that are located in bone, cartilage, fat, connective tissue, muscle, and other supportive tissues.
- Lymphomas are Cancers that begin in the lymph nodes and immune system tissues.

- ❖ Leukemias are Cancers that begin in the bone marrow and often accumulate in the bloodstream.
- ❖ Adenomas are Cancers that arise in the thyroid, the pituitary gland, the adrenal gland, and other glandular tissues [48].

Individual types of cancer

There are said to be over 200 different types of cancer. Some of the cancer types are as follows,

- Anal cancer
- Bladder cancer
- Bone cancer
- Breast cancer
- Cervical cancer
- Colon cancer
- Colorectal cancer
- Endometrial cancer
- Kidney cancer
- Leukemia
- Liver cancer
- Lymphoma
- Ovarian cancer
- Pancreatic cancer
- Prostate cancer
- Stomach cancer
- Testicular cancer
- Thyroid cancer
- Vaginal cancer
- Vulvar cancer

Metastasis:

Metastasis is the general term used to describe the spread of cancer cells from the primary tumor to surrounding tissues and to distant organs and is the primary cause of cancer morbidity and mortality. It is responsible for about 90% of cancer deaths. Metastasis involves a series of sequential and interrelated steps. Metastatic cells also establish a microenvironment that facilitates angiogenesis and proliferation, resulting in macroscopic, malignant secondary tumors. Although systemic metastasis is responsible for about 90% of cancer deaths. [49]

Head and neck Cancer:

Tumours of the head and neck are the sixth most common malignancy in the world, with a yearly incidence of more than 500,000 cases, and it comprises approximately 4% to 5% of all new Cancers and 2% of all Cancer deaths (100,000 per year).

Most patients are older than 50 years, and incidence increases with age; the male-to-female ratio is 2.5:1. Approximately 34% of oral and pharyngeal Cancers present as localized disease, 46% present as loco regional (i.e., locally advanced or involving regional lymph nodes) disease, and 10% present as metastatic disease. Ninety percent of these Cancers involve squamous cell histology.

The most common sites are the oral cavity, pharynx, larynx, and hypopharynx. Nasal cavity and paranasal sinus Cancers, salivary gland malignancies, and various sarcomas, lymphomas, and melanoma are less common.

Site-specific head and neck Cancers:

Oropharynx cancer

The subsites of the oropharynx are (1) the anterior surface of the soft palate, including the uvula; (2) the posterior pharyngeal wall; (3) the anterior and posterior tonsillar pillars; (4) the tonsils and tonsillar fossas; and (5) the posterior one-third of the tongue, which lies between the circumvallate papillae and the vallecula. The lingual surface of the epiglottis is part of the supraglottic larynx. The mucosa of the oropharynx consists of stratified squamous epithelium.

Pathology

Most oropharyngeal cancers are squamous cell carcinomas, Deep infiltration is common. Minor salivary gland cancers and lymphomas-particularly non-Hodgkin's lymphoma involving the tonsil and other parts of Waldeyer's ring-also arise in this area. Tumors of the parapharyngeal space may also present as oropharyngeal swellings, causing the mucosa of the lateral pharyngeal wall or soft palate to bulge. These are most often retromandibular parotid tumors or neurogenic tumors (eg, neurilemmoma, neurofibroma, or paraganglioma)

Cancers of the tonsillar region drain primarily to the upper and mid jugular chain nodes and to the submandibular nodes (levels I, II, and III). Posterior triangle lymph nodes (level V) become involved following involvement of the jugular chain nodes. The overall incidence of cervical lymph node involvement from cancer of the oropharynx is approximately 70%. Bilateral cervical lymph node metastases are common (50%).

Clinical Findings

Cancer of the oropharynx usually present with ulcerating cancers. Cervical lymph node metastases are a common presenting sign. Approximately 70% of patients with tongue base cancers present with advanced cancer, compared with only 35% of patients with cancer of the oral tongue. Tongue base cancers can spread laterally to involve the mandible, anteriorly to involve the oral tongue, and inferiorly to involve the vallecula and supraglottic larynx. Cancers of the tonsillar region readily extend to the mandible. They can also infiltrate the pterygoid muscles, producing trismus. This is an important presenting symptom, and it may limit access for examination. Referred otalgia is common with deeply infiltrating cancers.

B. Management of Neck Metastases

Cervical lymph node metastases are treated by radical or modified neck dissection. If the neck is clinically free of cancer, an elective supraomohyoid neck dissection or radiation therapy is appropriate. If the primary cancer is treated by radiation therapy, the cervical lymph nodes at risk are included in the radiation field. If the primary cancer is treated surgically, an elective neck dissection is appropriate. If postoperative radiation therapy is planned for the primary cancer site, it is reasonable to include the neck in the radiation field instead of performing an elective neck dissection. Initial (induction, neoadjuvant) chemotherapy using cisplatin, fluorouracil, and paclitaxel, singly or in combination, gives response rates of up to 80%, but these responses are rarely of long duration.

Hypopharynx

Over 95% of hypo pharyngeal cancers are squamous carcinomas, which usually present as infiltrating ulcerative lesions.Cancer of the hypopharynx has a high propensity for lymphatic invasion, with most patients having cervical lymph node metastases at the time of initial presentation.

The hypopharynx-especially the piri-orm sinus-must always be examined in an adult with cervical lymph node metastases and no obvious primary cancer site. Occult cervical lymph node metastases (ie, clinically negative but histologically positive) are also common, causing the overall incidence of cervical lymph node metastases at presentation to be approximately 75%.

Clinical Findings

The most common site for hypopharyngeal cancer is the piriform sinus, accounting for 60% of cases. The postcricoid region is affected in 25% of patients and the posterior pharyngeal wall in 15%.

The chief symptoms of hypopharyngeal cancer are pain, dysphagia, and weight loss. Pain can be localized to the site of the cancer or can be referred to the ipsilateral ear. Direct laryngopharyngoscopy and biopsy are necessary.

Salivary gland cancer

The paired major salivary glands consist of the parotid and submandibular glands. Minor salivary glands are widely distributed in the mucosa of the lips, cheeks, hard and soft palate, uvula, floor of mouth, tongue, and peritonsillar region; a few are found in the nasopharynx, paranasal sinuses, larynx, trachea, bronchi, and lacrimal glands.

Pathology

A variety of site-specific and systemic diseases can affect the salivary glands. Tumors of salivary gland tissue constitute about 5% of head and neck tumors and affect major salivary glands five times more often than minor salivary glands. The incidence of malignancy among salivary gland tumors varies inversely with the size of the gland. About 15% of parotid tumors, 50% of submandibular gland tumors, and 90% of minor salivary gland tumors are malignant.

Since 70% of salivary gland tumors occur in the parotid and 85% of these are benign, the majority of salivary gland tumors are benign. These tumors are thought to originate from two cell types: intercalated and excretory duct cells.

The most common benign salivary gland tumor is the benign mixed tumor or pleomorphic adenoma, which accounts for 70% of parotid tumors and 50% of all salivary gland tumors. Mixed tumors are more common in women than in men, with the peak incidence in the fifth decade. They are slow-growing and lobular and may become very large without interfering with facial nerve function. Although mixed tumors are benign, they will recur after surgery unless they are completely removed. Warthin's tumor (papillary cystadenoma lymphomatosum), the next most common benign tumor, accounts for about 5% of parotid tumors. Warthin's tumors are usually

cystic, typically occur in men in the sixth and seventh decades, and are bilateral in about 10% of cases. They occur almost exclusively in the parotid gland and have a typical histologic appearance, consisting of a papillary-cystic pattern with a marked lymphoid component. Oncocytomas are benign tumors composed of large oxyphilic cells called oncocytes.

Mucoepidermoid carcinoma is the most common parotid cancer. These tumors are categorized as high-grade, intermediate-grade, or low-grade cancers. Adenoid cystic carcinomas, which are uncommon in the parotid, have a great propensity for perineural invasion and local recurrence. Patients with this cancer tend to have protracted illness, with recurrences appearing 15 years or more after treatment. Approximately 70% of all minor salivary gland cancers occur in the oral cavity, principally on the hard palate. [50]

Oral Cancer:

Oral Cancer is one of the most common head and neck malignancies. Oral Cancer is a general term for oral cavity Cancers. It occurs in the majority, where squamous cell carcinoma, which is called the mucosa mutate. In clinical practice, oral Cancer, including Cancer gums , tongue , hard and soft palate, carcinoma of the mandible , oropharynx, lip Cancer and maxillary sinus. Cancer occurs in the facial skin and mucous membranes of oral cavity and so on.

Causes of Oral Cancer:

- Long-term habit of tobacco, alcohol
- Poor oral hygiene:
- Long-term stimulation by foreign body:
- Malnutrition:

Vitamin A deficiency can cause oral mucosal epithelial thickening, hyperkeratosis with the occurrence of oral Cancer. Demographic studies show that countries with low intake of vitamin A high incidence of oral Cancer. There is also an inadequate intake of trace elements considered relevant, such as low Zinc content of foods. Zinc is indispensable for the growth of animal tissue elements, Zinc deficiency may lead to mucosal epithelial damage, and create favorable conditions for the occurrence of oral

Cancer. Inadequate plant protein and animal protein intake may be associated with oral Cancer.

There are **four stages** of oral cancer.

- **Stage 1:** The tumor is 2 centimeters (cm) or smaller, and the cancer hasn't spread to the lymph nodes.
- **Stage 2:** The tumor is between 2-4 cm, and cancer cells haven't spread to the lymph nodes.
- **Stage 3:** The tumor is either larger than 4 cm and hasn't spread to the lymph nodes, or is any size and has spread to one lymph node, but not to other parts of the body.
- **Stage 4:** Tumors are any size and the cancer cells have spread to nearby tissues, the lymph nodes, or other parts of the body [51].

Associated lesions:

The relationship between oral Cancer and precancerous lesions:

White ulcers or blisters inside the buccal mucosa occurs, often occurs as pressure sores, poor sleep or eating habits (such as insufficient fruits), in general will heal within two weeks. If it is not cured in two weeks, must be examined to rule out the possibility of epithelial cell carcinoma.

Changes in the oral mucosa color:

Normal epithelium pink, red or a white color of polarization is not normal. If red with white, it is more serious situation, another example of the tongue appears dark red with white dot like, highly suspicious of Cancer.

Ulcer:

Over more than two weeks of oral mucosal ulcer has not yet healed.

Clinical manifestations:

- Lumps, nodules
- White, smooth scaly plaque appeared
- Red patches, ulcers, inflammation and other symptoms distinct can't be cured for a longer period
- Repeated oral bleeding for no apparent reason

- ❖ Numbness, burning, or dryness of mouth for no apparent reason
- ❖ Unusual difficulty in speaking or swallowing [52].

Diagnosis:

When neck mass is the first presentation, the primary site can be located and biopsied in approximately 80% of cases. If no primary site is obvious, tissue diagnosis can be obtained by fine needle aspiration (**FNA**) biopsy of the node, with sensitivity and specificity approaching 99%. A non-diagnostic FNA does not rule out the presence of tumour.

Computerized tomography scan (**CT scan**) remains the primary imaging study for evaluation of metastatic adenopathy. Magnetic resonance imaging (**MRI**) may complement the CT scan. Positron emission tomography **(PET**) scans are being used more frequently to detect tumours that are not obvious on other scans.

Laryngoscopy and **nasopharyngoscopy** should be performed. With occult primary tumours, directed biopsies of the nasopharynx, tonsil, base of tongue, and pyriform sinus should be performed. Bilateral tonsillectomy will sometimes reveal the source of an occult Cancer

Panoramic radiograph.

This is a rotating, or panoramic, x-ray of the upper and lower jawbones to detect cancer or evaluate the teeth before radiation therapy or chemotherapy. This is often called a Panorex.

Bone scan

A **bone scan** uses a radioactive tracer to look at the inside of the bones. The tracer is injected into a patient's vein. It collects in areas of the bone and is detected by a special camera. Healthy bone appears gray to the camera, and areas of injury, such as those caused by cancer, appear dark. This test may be done to see if cancer has spread to the bones.

Prevention:

1. Avoid unnecessary prolonged illumination, prevent the emergence of lip Cancer
2. Avoid smoking and drinking
3. Patients wearing dentures

a. Found that tissue under dentures pain, inflammation, should seek immediate medical attention. Strive to achieve early detection of Cancer, early diagnosis and early treatment, and insisted regularly checked.

4. Balanced diet
5. Do not drink and eat hot water and food, to avoid irritation of oral tissues
6. Unplug the tooth residual root and crown
7. Good wear dentures, does not stimulate tissue
8. Develop good oral hygiene habits
9. Regular brushing. Pay attention to nutritional balance, timely treatment

Four symptoms of oral Cancer to be alert:

If the mouth turns white, brown or black, it means a change in mucosal epithelial cells. Especially the oral mucosa becomes rough, thickened or showed induration, appeared oral leukoplakia , erythema, is likely to be Cancerous.

Unhealed ulcer:

Oral ulcers of the course is generally not more than two weeks, if the burning sensation, pain and other symptoms brought the matter still more than two weeks, to be alert to the possibility of oral Cancer.

Obvious pain:

Intially generally painless or only partial exception sense of friction, ulceration obvious pain, with further violations of the nerve tumor, can cause ear and throat pain.

Lymph nodes:

Multiple oral Cancer to nearby lymph node metastasis to the neck, and sometimes the primary lesion is small, and even the symptoms are not obvious, but they found a lymph node metastasis of Cancer cells. Therefore, such a sudden neck lymph node, need to check the mouth.

Dysfunction:

Zhang closed the tumour may infringe muscles and temporomandibular joint, resulting in the opening and closing movement is restricted

Cervical cancer

Cervical cancer is the most common cancer in women aged 35 and below. Cervical cancer is a type of cancer that occurs in the cells of the cervix. The cervix is the organ connecting the uterus and vagina. It is usually a slow- growing cancer that may not have symptoms but can be found with regular pap tests.

This is a procedure in which cells are scraped from the cervix and looked at under a microscope [53].

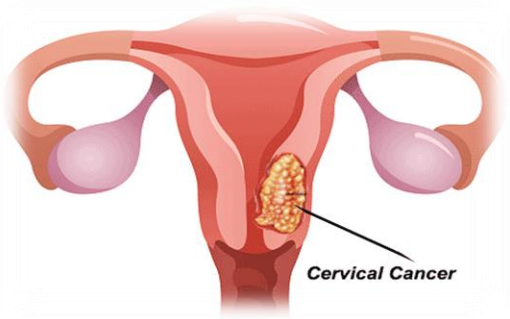


Figure 9 Cervical cancer

Cervical cancer is not thought to be hereditary. In 99.7% of cases, cervical cancers are caused by persistent infections with a virus called high-risk human papillomavirus (HPV). HPV is a very common virus transmitted through skin to skin contact in the genital area. Around four out of five sexually active adults (80%)

will be infected with some type of HPV in their lives. While HPV infection is common, cervical cancer is rare [54]. There are two types of cervical cancer. Squamous cell carcinomas (80% to 90%) and adeno carcinomas (5% to 20%). If the cancer has signs of both types, it is called mixed carcinoma. Early detection and treatment of cervical cancer is important to improve survival.

Predisposing Factors [56]

- Average age 35-45 years

- Coitus before the age of 18 years
- Multiple sexual partners
- Delivery of the first baby before the age of 20 years
- Multiparty with poor birth spacing between pregnancies.
- Poor personal hygiene
- Poor socioeconomic status
- Previously, exposure to smegma from uncircumcised partners was considered an important for lower incidence of cancer cervix amongst the Jews and Muslims.
- Smoking and alcohol
- Contraceptive pill- long term use of some common contraceptive pills slightly raises a women's risk.

History of Cervical Cancer [55]

Table 5. History of Cervical Cancer

Year/ Period	***Key developments***
19th century	Cervical cancer is identified as a sexually transmitted disease. At the end of the century, surgery is introduced for treating the disease.
Early 20th century	Epidemiologists discover that cervical cancer is common in female sex workers and also common in women whose husbands have a high number of sexual partners or were regular customers of prostitutes.
1920s	Papanikolaou develops his eponymous technique. The colposcope is developed.
1940s	Pap smear screening begins.
1980s	First concrete evidence that specific Human Papillomavirus (HPV) types are linked to cervical cancer. Tobacco use is linked to cervical cancer.
2000s	First Human papilloma virus (HPV) vaccine is released. Several nations introduce the vaccination, such as United States, Canada, Australia and Japan.
Recent years	Today, cervical cancer is both the fourth-most common cause of cancer and the fourth-most common cause of death from cancer in women. In 2012, approximately 528,000 cases of cervical cancer occurred, with 266,000 deaths. This is about 8% of the total cases and total deaths from cancer. About 70% of cervical cancers occur in developing countries.

- Women with STD, HIV infection, herpes simplex virus 2 infection, HPV infection (16, 18, 31, 33) or condylomata have a high predisposition to cancer.
- Immuno suppressed individuals (following transplant surgery)
- Women with pre invasive lesions
- Women who do not come for regular health check-up and pap test.

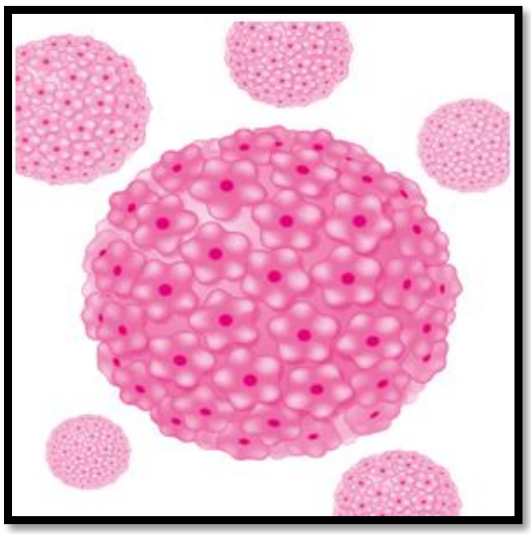


Figure 10. Show Human papilloma virus

Human papillomavirus (HPV)

Human papilloma virus (HPV) is an extremely common virus. HPV is the most widespread of all sexually transmitted viruses. four out of five (80%) of the world's population will contract some type of the virus once in their life. There are over 100 identified types of HPV and each different type has been assigned a specific number.

The majority of HPV types infect the skin on external areas of the body, including the cause verrucas on the feet. hands and feet.

- Around 40 of the HPV types affect the genital areas of men and women including the skin of the penis, vulva, anus and the linings of the vagina, cervix and rectum.
- Around 20 of these types are thought to be associated with the development of cancer. The World Health Organization (WHO) International Association for Research on Cancer (IARC) defines 13 of these 20 types as oncogenic (cancer causing).

These high-risk types of HPV are

- HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. A person infected with a high-risk genital HPV will show no symptoms.
- In addition, there are nine HPV types that may also be associated with the development of cervical cancer, these are HPV 26, 53, 64, 65, 66, 67, 69, 70, 73 and 82.

- The remaining genital HPV types have been designated low risk as they do not cause cervical cancer but they can cause other problems, such as genital warts [57].

Pathology

Pap smear in invasive cancer shows tadpole cells, haemorrhage and necrosis in the background. It is customary to identify two types of cancers of the cervix. The first and more common variety is the epidermoid carcinoma. It arises from the stratified squamous epithelium of the cervix and accounts for almost 80% of all cancers in the cervix.

The second variety endocervical carcinoma arises from the mucous membrane of the endocervical canal, accounts for 20% of all cervical cancers. Histologically, 95 percent of cervical cancers are squamous carcinomas and only 5 percent are adenocarcinoma.

This is because the columnar epithelium of the endocervix often undergoes squamous metaplasia. Endocervical cancers of the cervix have recently increased in incidence because of prolonged use of oral combined contraceptive pills and progesterone's which have profound effect on glandular epithelium.

The malignant cells are endometroid, adenocarcinoma, clear cells and adenosquamous. Squamous cell cancers of the ectocervix appear as proliferative growths, ulcers or as flat indurated areas. The common proliferative or cauliflower-like growth is vascular, friable and bleeds on touch.

It undergoes ulceration and necrosis, which is associated with an offensive foul smelling vaginal discharge. The leucorrhoeal discharge is often blood stained. Histologically, the tumor is graded as well-differentiated or ill-differentiated. The endocervical growth remains confined to the cervical canal for a long time causing a barrel-shaped enlargement of the cervix, and only at a late stage it protrudes beyond the external cervical os and becomes visible.

The mode of spread of the cancer is by continuity or by continuity, by lymphatic spread or through vascular embolism to distant sites like lungs, liver, bones, kidneys and brain. Ovarian metastasis occurs in only 1 percent

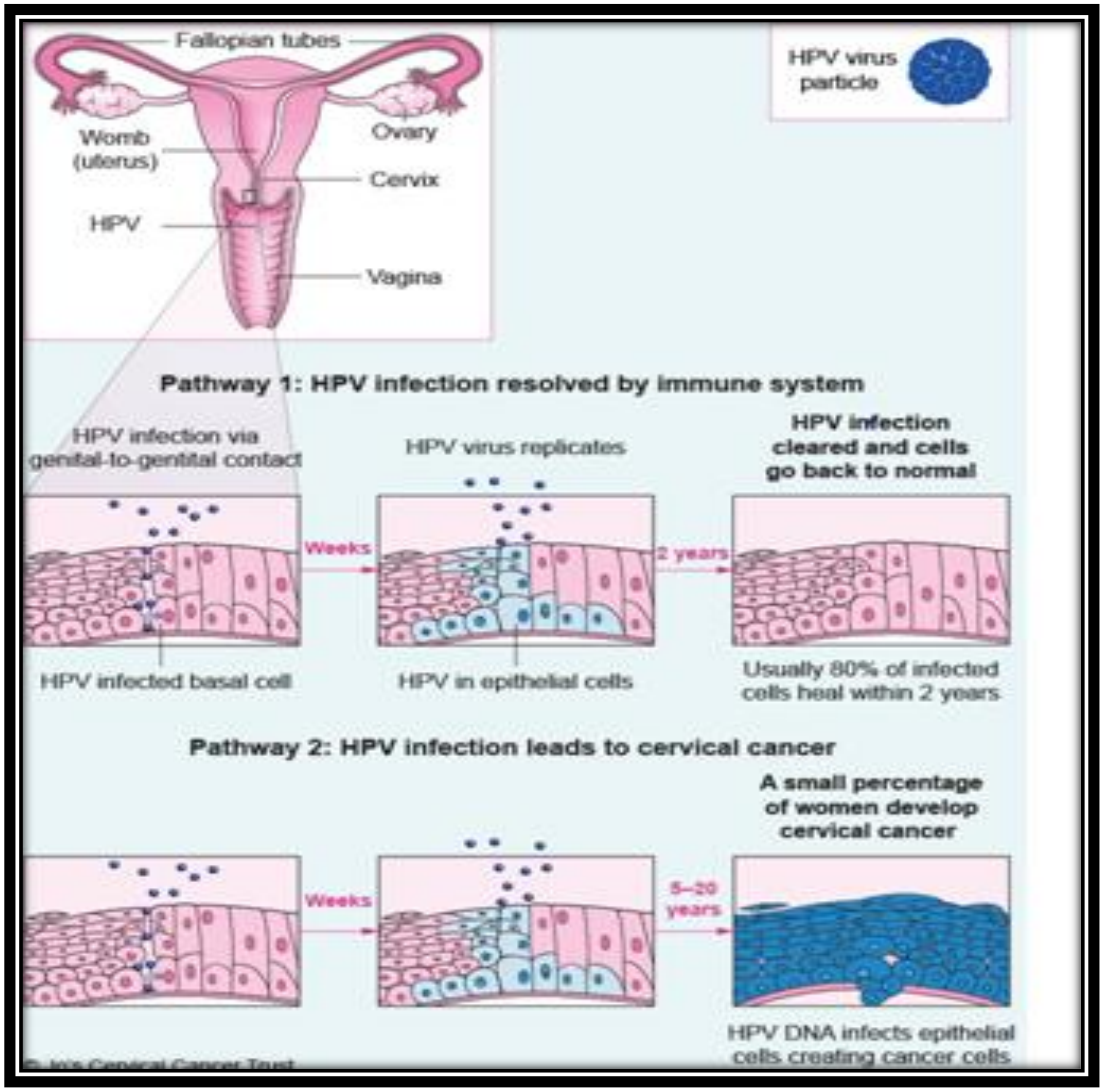


Figure 11. HPV infection

Stages of Cervical cancer

Stage I

- Carcinoma strictly conflicted to the cervix IA Micro invasive carcinoma, not exceeding 5.0mm.
- IA1 Measured stromal invasion of less than 3.0mm in depth.
- IA2 Measured stromal invasion between 3 and 5mm in depth.
- IB Clinically visible lesion confined to the uterus

- ❖ IB1 Clinically visible lesion 4.0cm.
- ❖ IB2 Clinically visible lesion more than 4.0cm in dimension.

Stage II

- ❖ Cancer spread beyond the cervix, but not to pelvic wall or lower third of the vagina.
- ❖ IIA Tumor without parametrical invasion.
- ❖ IIB Tumor with parametrical invasion.

Stage III

- ❖ Tumor extends to the lateral pelvic wall, involves the lower third of vagina, and/or causes hydronephrosis or nonfunctioning kidney.

Stage IV

- ❖ Tumor spread to the pelvic organs or distal metastasis.
- ❖ IVA Tumor involves bladder and rectum.
- ❖ IVB Widespread tumor with distant metastasis.

Differential Diagnosis:

- ➢ Tubercular ulcer
- ➢ Syphilitic ulcer
- ➢ Fibroid polyp
- ➢ Sarcoma of the cervix

Diagnosis [58]

The earlier cervical cancer is diagnosed the more successful treatment can be done. Regular cervical screening can save thousands of lives every year.

- ➢ Cervical smear test
- ➢ HPV DNA Test

Additional tests including

- ➢ Biopsy
- ➢ Colposcopy
    1. Punch biopsy
    2. Endocervical curettage
- ➢ Cone biopsy, LLETZ

- Blood tests
- Examination under anesthesia (EUA)
- CT scan, MRI, Pelvic ultrasound

Treatment

The management of patients with head and neck Cancer is complex. The choice of treatment modality depends on the stage and site of disease. Patients with locally advanced disease should be evaluated (prosthodontics, nutrition, speech, and swallowing) by a multidisciplinary team including otolaryngologist or head and neck surgical oncologist, radiation oncologist, medical oncologist, dentist, and personnel involved in rehabilitation before treatment is initiated. In general, either surgery or radiation is effective as single-modality therapy for patients with early-stage disease (stage I or II) for most sites. The choice of modality depends on local expertise, patient preference, and functional result. For the 60% of patients with locally advanced disease (stage III, IV, and M0), combined-modality therapy is indicated [59].

Surgery

The nature of the surgical procedure is determined primarily by the size of the tumour and the structures involved. Extensive surgeries and those involving function of the tongue. Frequently require mucocutaneous flaps or microvascular free flaps to achieve a more functional reconstruction.

Resectability depends on the experience of the surgeon and the rehabilitation team. In general, a tumour is unresectable if the surgeon believes that all of the gross tumour cannot be removed or that local and distant control will not be achieved after surgery even with adjuvant radiation therapy. Generally, involvement of the skull base, pterygoid, and deep neck musculature, and of the major vessels portends a poor outcome with surgery as a primary modality.

Cervical lymph node dissections may be elective or therapeutic. Elective neck dissections are done at the time of surgery in patients with necks that are clinically negative when the risk of a positive lymph node is at least 30%. Therapeutic neck dissections are done for clinically obvious masses. This surgery is now rarely performed because of excessive morbidity, especially loss of shoulder function. The modified radical dissection preserves one or more of the non-lymphatic structures. In selective

neck dissections, only certain levels of lymph nodes are removed on the basis of the specific lymphatic drainage from the primary site.

Radiation therapy [63]

Radiation therapy uses high energy x-rays or radioactive particles to kill cancer cells. Radiation therapy may be used for cervical cancer.

The two types of radiation therapy most often used to treat cervical cancer include:

1. External beam radiation
2. Brachytherapy

1.External beam radiation

One way to give radiation is to aim x-rays at the cancer from outside the body. This is called External beam radiation therapy **(EBRT).** Each radiation treatment lasts only a few minutes, but getting you into place for treatment usually takes longer. The procedure itself is painless.

When radiation is used as the main treatment for cancer, EBRT is usually combined with chemotherapy (called concurrent chemoradiation). Often, a low dose of the chemo drug called cisplatin, but other chemo drugs can be used as well. The radiation treatments are given 5 days a week for a total 6 to 7 weeks. The chemotherapy is given at scheduled times during the radiation. The schedule is determined by which drug is used. EBRT can also be used by itself to treat areas of cancer spread or as the main treatment of cervical cancer in patients who can't tolerate chemoradiation.

Possible side effects of EBRT

Side effects of external beam radiation therapy for cervical cancer can include:

- Fatigue (tiredness)
- Upset stomach
- Diarrhea or loose stools (if radiation is given to the pelvis or abdomen)
- Nausea and vomiting
- Skin changes
- Radiation cystitis
- Vaginal pain
- Menstrual changes

- Low blood counts

1.Brachytherapy

Brachytherapy or internal radiation therapy, puts a source of radiation in or near the cancer. This type of radiation only travels a short distance. The type of brachytherapy used most often to treat cervical cancer is known as **intracavitary brachytherapy**. The radiation source is placed in a device in the vagina (and sometimes in the cervix). This is often used in addition to EBRT as a part of the main treatment for cervical cancer.

There are two types of brachytherapy:

- Low-dose rate (LDR) brachytherapy
- High-dose rate (HDR) brachytherapy

Possible short-term side effects of brachytherapy

Since the radiation only travels a short distance with brachytherapy, the main effects of the radiation are on the cervix and the walls of the vagina. The most common side effect is irritation of the vagina. It may become red and sore and there may be a discharge. The vulva may become irritated as well.

Brachytherapy can also cause many of the same side effects as EBRT, such as fatigue, diarrhea, nausea, irritation of the bladder and low blood counts.

Long term side effects of radiation therapy

- Vaginal stenosis
- Vaginal dryness
- Weakened bones
- Swelling of the legs

Chemotherapy:

Until relatively recently, chemotherapy was used mainly for palliation of patients with locally recurrent or disseminated disease without proven survival advantage. Combination chemotherapy yields higher response rates but has increased toxicity and no proven survival advantage when compared with single agents. The choice of single-agent or combination chemotherapy depends on the patient's preference and

performance status. The combination of Cisplatin and infusional 5-Fluorouracil (5-FU) produces a 70% response rate and a 27% complete remission (CR) rate in chemotherapy-naive patients.

Platinum-based chemotherapeutic regimens and the single agent methotrexate are the most commonly used regimens for metastatic disease. Carboplatin may be slightly less active than Cisplatin for head and neck squamous Cancer, but Carboplatin combinations with other chemotherapy agents are generally better tolerated than those with Cisplatin.

Both Docetaxel and Paclitaxel have shown antitumor activity. Several dosing schedules for Paclitaxel have been investigated. Three-hour infusions are probably the best balance between theoretically optimum exposure and tolerable toxicity. Docetaxel is usually administered at doses of 60 to 100 mg per m^2 every 3 to 4 weeks.

The role of chemotherapy has expanded significantly over the last decade because of the results of clinical trials incorporating chemotherapy in multimodality regimens for previously untreated disease.

Induction Chemotherapy:

Induction chemotherapy followed by definitive radiation therapy in patients responding to chemotherapy has been studied for organ preservation in patients with locally advanced Cancers of the larynx and of the hypopharynx. No significant survival difference has been demonstrated for chemotherapy followed by radiotherapy compared to surgery followed by radiotherapy in these patients. For laryngeal Cancer, concomitant Cisplatin and radiation therapy leads to better local control. Presently, induction chemotherapy followed by radiation therapy can be considered standard only for patients with previously untreated locally advanced squamous Cancers in the hypopharynx.

Concomitant chemoradiation:

The rationale for concomitant chemoradiation is based on experimental evidence of synergism between chemotherapy and radiation that is theoretically mediated by interference of chemotherapy with multiple intracellular radiation-induced stress-response pathways involved in apoptosis, proliferation, and DNA repair.

The finding that certain chemotherapeutic agents (e.g., Cisplatin, 5-FU, Taxanes, and Hydroxycarbamide) can induce radiosensitivity and increase log cell kill for radiation supports this treatment strategy.

Cisplatin, the most extensively evaluated drug in recent large randomized trials, has the advantage of not having mucositis as toxicity, although as a radiation enhancer, it does increase radiation-induced mucositis.

Adjuvant Chemotherapy:

A large randomized study in resected patients with stage III or IV disease compared adjuvant radiation therapy with adjuvant chemotherapy followed by radiation. Patients with low-risk disease did not benefit from adjuvant chemotherapy.

Adjuvant concomitant cisplatin and radiation in patients at high risk for recurrence after surgery has been studied both in Europe and in the United States. Both studies found a possible benefit in disease-free or overall survival for patients receiving concomitant cisplatin and radiation [60].

As a part of the main treatment for cervical cancer

For some stages of cervical cancer, the preferred treatment is radiation and chemo given together (called concurrent chemoradiation). The chemo helps the radiation work better.

Options for concurrent chemoradiation include:

- Cisplatin given weekly during radiation. This drug is given into a vein (IV) about 4 hours before the radiation appointment.
- Cisplatin plus 5-fluorouracil (5-FU) given every 4 weeks during radiation.
- Sometimes chemo is also given (without radiation) before and/or after chemoradiation

Side effects of chemotherapy

Chemo drugs kill cancer cells but also damage some normal cells, which can lead to certain side effects. Side effects depend on the type and dose of the drugs and the length of time you are treated.

- Nausea and vomiting
- Loss of appetite

- Loss of hair
- Mouth sores
- Fatigue (tiredness)

Because chemotherapy can damage the blood-producing cells of the bone marrow. This can result in:

- An increased chance of infection (from a shortage of white blood cells)
- Bleeding or bruising after minor cuts or injuries (because of a shortage of blood platelets)
- Shortness of breath (due to low red blood cell counts)

When chemo is given with radiation, the side effects are often more severe. Nausea, fatigue and problems with low blood counts are often worse. Diarrhea can also be worse if chemo is given at the same time with radiation and the following side effects can occur,

- Menstrual changes
- Neuropathy
- Increased risk of leukemia

Other side effects are also possible. Some of these are more common with certain chemo drugs. Many side effects are short-term and go away after treatment is completed, but some can last for a longer time or even be permanent.

Targeted therapy / Immunotherapy [61]

- Targeted therapy is a type of treatment that uses drugs or other substances to identify and attack specific cancer cells without harming normal cells.
- Monoclonal antibody therapy is a type of targeted therapy that uses antibodies made in the laboratory from a single type of immune system cell.
- Bevacizumab is a monoclonal antibody that binds to a protein called vascular endothelial growth factor (VEGF) and may prevent the growth of new blood vessels that tumors need to grow. Bevacizumab is used to treat cervical cancer that has metastasized (spread to other parts of the body) and recurrent cervical cancer.

Vaccines:

A number of infectious a gents cause Cancer. Hepatitis B and C are linked to liver Cancer, some human papilloma virus (HPV) strains are linked to cervical and head and neck Cancer, and Helicobacter pylori is associated with gastric Cancer and lymphoma. Vaccines to protect against these agents may reduce the risk of their associated Cancers. The hepatitis B vaccine is effective in preventing hepatitis and hepatomas due to chronic hepatitis B infection. Public health officials are encouraging widespread administration of the hepatitis B vaccine, especially in Asia, where the disease is epidemic.

A four-valent HPV vaccine (Gardasil) is 100% effective at preventing infection. The vaccine is recommended for girls and women ages 9–26 years. Reduction in these HPV types could prevent >70% of the cervical Cancers worldwide [62]

Cervix

Produced by GlaxoSmithKline, is designed to protect against HPV types 16 and 18 only Both vaccines are licensed in the UK. The NHS currently uses Gardasil to vaccinate girls.

- There is also a third vaccine called **Gardasil 9.**
- This protects against high risk HPV types 16, 18, 31, 33, 45, 52 and 58, as well as HPV 6 and 11. Gardasil 9 has been approved for licensing by the Committee for Medicinal Products for Human use of the European Medicines Agency.
- The vaccination is free for all girls from the age of 11 in Scotland and 12 in the rest of the UK up to their 18th birthday, but only girls aged 11 to 13 in Scotland and 12 to 13 in the rest of the UK will be routinely offered the vaccine.
- The vaccination is given to girls at this age because their immune systems are at their strongest before puberty begins and the vaccination works best when the immune system is strong.
- Both Gardasil and Cervix protect against the two highest risk HPV types. However, unfortunately, women can be infected with more than one type of HPV. Having the vaccine will provide protection against 70% of all cervical

cancers and it will also prevent most of the more serious precancerous cervical changes (classed as moderate or severe cervical abnormalities).

Prevention of cervical cancer [60]

There are a number of measures that can be taken to reduce the chances of developing cervical cancer.

- Human papillomavirus (HPV) vaccine
- Safe sex and cervical cancer
- Cervical screening, Having fewer sexual partners
- Delaying first sexual intercourse

3.3 PHARMACOLOGICAL REVIEW

Our great *Siddhars* explained many medicinal preparations to cure the life threatening Cancer disease and a few medicines for cancer are listed here,

Herbal origin:

Compound herbal preparations

Pills:

- Chithiramoolaakuligai [64]
- Maha Kodasoozhi Mathirai
- Aswaganthathi vadagam

Chooranam

- Vallathy Chooranam
- Megaroga Chooranam
- Garudakodi Chooranam
- Sorkkamara Chooranam
- Karanthai Chooranam[65]

Mineral and metal origin:

Siddhars identified and worked on many metal and mineral preparations which had anti-cancer activity**.**

Parpam:

- Thambira Parpam [66]

- Gandhaga Poora Parpam [67]
- Kariya Parpam
- Rasa Parpam [68]
- Naga Parpam [69]
- Thalaga Parpam
- Sandarasa Parpam
- Sootha Parpam [86]

Chendhooram:

- Panchapadana Chendhooram [70]
- Swaranapushpa Rasa Chendhooram [62b]
- Muthu Chendhooram [71]
- Kandhaga Chendhooram
- Gowri Chinthamani Chendhooram
- Linga Chendhooram
- Rasa Chendhooram[71a]
- Thambira Chendhooram
- Pavala Vanga Chendhooram [70 b]
- Kalameganarayana Chendhooram [72]
- Navachara Chendhooram [73]
- Naga Chendhooram [74]
- Namachivaya Chendhooram [75]
- Narayana Chendhooram [76a]

Nei:

- Chitramoola Nei [77]
- Kukkil Nei [78a]
- Thengai Nei
- Vallarai Nei

Ennai:

- Perungaya Ennai [82]
- Singi Thylam [79a]
- Mega Santhanathy Thylam
- Chinthamani Ennai

- Meganathi Ennai
- Visha Rajanga Thylam
- Pachai Thylam [79 b]
- Sengathari Thylam [80a]
- Mega Rasanga Ennai [80b]
- Puda Thylam [78b]
- Gandhaga Thylam (74b)
- Sengottai Thylam
- Vipuruthi thylam[82]

Mezhugu:

- Rasagandhi Mezhugu[67]
- Kanaga Linga Mezhugu [83a]
- Vaalai Rasa Mezhugu
- Gandhaga Mezhugu (84)
- Korasanai Mezhugu
- Veera Mezhugu [74a]

Kattu:

- Poorakattu (85)

Pathangam:

- Lingapathangam (83c)
- Gurupathangam [83b)
- Putru pathangam
- Veera Rasa pathangam [87]

Others:

- Rana Pugai [80b]
- Veelai seelai [80c]
- Kulirntha pachai
- Chithiravallathi Legium
- Veelai seelai [88]
- Kandharasa villai

Siddha drugs for Yoniputru

Pills:

- Chitramoola Kuligai

Chooranam:

- Karanthai chooranam

Parpam:

- Thambira Parpam
- Velli Parpam
- Gandhaga Parpam
- Karuvanga Paarpam
- Rasa parpam

Chendhooram:

- Muthu Chendhooram
- Namachivaya Chendhooram
- Swarnapushpa Rasa Chendhooram
- Kalamega narayana chendhooram
- Pancha padana chendhooram
- Muthu chendhooram

Thylam:

- Chitramoola Nei
- Vallarai Nei [79c]
- Megathu Ennai
- Gandhaga Thylam
- Sathrusangara Ennai
- Perungaya Ennai
- Meganathi Thylam

Mezhugu:

- Vithu Rasa Mezhugu [76b]
- Korosanai Mezhugu
- Rasaganthi Mezhugu
- Gandhaga Mezhugu

- Amirtha Nandhi Mezhugu
- Korosonai mezhugu
- Markandeya Mezhugu
- Korosanaimezhu

Kattu:

- Poora Kattu

Pathangam:

- Linga pathangam
- Guru pathangam
- Putru pathangam [90]

Kirutham:

- Vallarai kirutham [76 b]

Others:

- Gandha Rasa Villai [74c]
- Megam pokkum Rasagandhi [86b]
- Soolai Kudori [74d]
- Rana pugai [89]

ANTICANCER DRUGS- MODERN ASPECT

The drugs which prevent neoplasm are known as anticancer drugs. They also called antineoplastic drugs

They may be divided into two classes

- Cycle specific

Cycle specific drugs act only at specific points of the cell's duplication cycle, such as anaphase or metaphase

- Non- cycle specific

Drugs may act any point in the cell cycle, in order to gain maximum effect, anti-neoplastic drugs are commonly used in combinations.

Classification [91]

1. Alkylating agent
2. Antimetabolites
3. Natural origin
   From plants
   From micro organisms
4. Hormones
5. Miscellaneous

Precautions

Because antineoplastic agents do not target specific cell type, they have a number of common adverse side effects.

- Hair loss is common due to the effects on hair follicles
- Anaemia
- Immune system impairment
- Clotting problem caused by destruction of the blood forming organs, leading to reduction in the number of red cells, white cells and platelets.

Interactions

Anticancer drugs may interact with a number of other medicines. When this happens, the effects of one or both of the drugs may change or the risk of side effects may be greater.

Table 6. Anticancer Drugs [92]

Generic (Brand Name)	Clinical Uses	Common Side Effects To Drug
Cisplatin (Platinol)	Treatment of head and neck cancer, bladder, uterine, testicular, ovarian cancer.	Renal toxicity and ototoxicity
Fluorouracil (Adrucil)	Treatment of breast cancer, head and neck cancer, colorectal cancer, cervical stomach cancer, pancreatic cancer.	CNS damage, cardio toxicity, anaphylaxis, encephalopathy

Altretamine (Hexalen)	Treatment of advanced ovarian Cancer	Bone marrow depression, nausea and vomiting
Asparaginase (Elspar)	Commonly used in combination with other drugs; refractory acute lymphocytic leukemia	Liver, kidney, pancreas, CNS abnormalities,
Bleomycin (Blenoxane)	Lymphomas, Hodgkin's disease, testicular cancer	Hair loss, stomatitis, pulmonary toxicity, hyperpigmentation of skin.
Carboplatin (Paraplatin)	Palliation of ovarian cancer	Bone marrow depression, nausea and vomiting
Carmustine	Hodgkin's disease, brain tumours, multiplemyeloma, malignant melanoma	Bone marrow depression ,nausea and vomiting
Chlorambucil (Leukeran)	Chronic lymphocytic leukemia, non-Hodgkin's lymphomas, Breast and ovarian cancer	Bone marrow depression, excess uric acid in blood
Cyclophosphamide (Cytoxan)	Hodgkin's disease, non-Hodgkin's lymphomas, neuroblastoma.ovarian, and lung cancer, acute lymphoblastic leukemia in children	Bone marrow depression, hair loss, nausea and vomiting, inflammation of the bladder.
Cytarabine (Cytosar- U)	Leukemias occurring in adult and children	Bone marrow depression, nausea &vomiting,diarrhoea, stomatitis
Methotrexate (Trexall, Rheumatrex)	Breast cancer, lung cancer, leukemia, osteosarcoma	Hepatotoxicity, renal failure, myelopathy
Melphan	Multiple myeloma,Ovarian cancer	Bone marrow depression, Infections,diarrhoea,pancreatitis

Diethylstilbestrol (DES) (Stilbestrol)	Breast cancer in post-menopausal women, prostate cancer	Hair loss, nausea and vomiting, oedema, excess calcium in blood; feminizing effects in men
Ethinyl estradiol (Estinyl)	Advanced breast cancer in post-menopausal women, prostate cancer	Excess calcium in blood, anorexia, oedema, nausea and vomiting; feminizing effects in men
Mitomycin (Mutamycin)	Bladder, breast, colon, lung, pancreas, rectum cancer, head and neck cancer, malignant melanoma	Bone marrow depression, nausea and vomiting, diarrhoea, stomatitis, possible tissue damage
Mitoxantrone (Novantrone)	Acute nonlymphocytic leukemia	Cardiac arrhythmias, laboured breathing, nausea& vomitting
Paclitaxel (Taxol)	Advanced ovarian cancer	Bone marrow depression, hair loss, nausea& vomiting, hypotension, allergic reactions, slow heart action, muscle and joint pain.
Pentastatin (Nipent)	Hairy cell leukemia unresponsive to alpha-interferon	Bone marrow depression, fever, skin rash, liver damage, nausea and vomiting
Prednisone (Meticorten)	Used in adjuvant therapy palliation of symptoms in lymphomas, acute leukemia Hodgkin's disease	May be toxic to all body systems
Streptozocin (Zanosar)	Islet cell carcinoma of pancreas	Nausea and vomiting, toxicity to kidneys
Tamoxifen (Nolvadex)	Advanced breast cancer in post-menopausal	Nausea and vomiting, ocular toxicity, hot flashes

Teniposide (Vumon)	Acute lymphocytic leukemia in children	Bone marrow depression, nausea and vomiting, hair loss.
Vinblastine (Velban)	Breast cancer, Hodgkin's disease, metastatic testicular cancer	Bone marrow depression, neurotoxicity
Ifosfamide	Head and cancer, bronchogeniccancer ccancer,bronchogenic,breast,testicular,bladder,osteogenic carcinoma, some lymphomas.	Haemorrhagic cystitis,less alopecia,less emetogenic.
Decarbazine(DTIC)	Malignent melanoma, Hodgkins disease	Nausea,vomiting,Flu like sysmptoms,neuropath and mylosuppression
Procarbazine	Hodgkin's disease,brain tumour	Males sufferm from sterility,vomiting,leucopenia,thrombocytopenia.
Fludarabine	Chronic lymphatic leukemia,non Hodgkins lymphoma,	Fever,chills,myalgia,artharalgia and vomiting,mylosupression.
Estramustine	Metasttic prostate cancer,	Myelo supression and estrogenic adverseeffects.viagynaecomstia,impotence,fluidretention,angioedema.

PHARMACOLOGICAL REVIEW OF CANCER:

Cancer is one of the thrust area for which effective drugs at comfortable prices are not available as yet probably due to lack in understanding the cancer Patho physiology. For such a dreadful disease anti-cancer drugs have been developed from a variety of sources ranging from natural products (plants and

microbes) to synthetic molecules.

The pharmacological screening of plants, minerals and animals is an essential mean for the invention of new, harmless and effective drugs. Over 50,000 plants have therapeutic virtues in the world, and around 80% of human use medicines based on plants and salts at least once in their life, Medicinal plants and mineral shave diversified chemical constituents which are important for the discovery of new active molecules against many types of cancer.

Nowadays it has become mandatory to monitor the quality of life of patients while in treatment of cancer. It is healthy aware that the quality of life of cancer patients treated with chemotherapeutic drugs are very much affected even long time after withdrawal of drugs. Therefore, the challenging task at this moment is to identify the quick and novel methods that can identify and develop molecules, which can be of therapeutic value in human cancers.

For this purpose, both, the *in vitro* and *in vitro* models are employed for systematic screening of an anticancer drug. This necessitates screening of a large number of compounds. For this purpose, both in-vitro and in-vivo models are employed for systematic screening of an anticancer drugs. [93]

INVITRO METHODS:

In studies in vitro cytotoxicity on cell line, various cell staining methods are used in order to indirectly estimate the number of viable cells present after treatment. An ideal test in assessing cell proliferation and cytotoxicity should have as main feature in vitro: be simple, fast, efficient, economical, reproducible, sensitive, safe, and effective as far viable cell population and do not show interference with to evaluate the compound.

ADVANTAGES [94]:

- Reduce the usage of animals
- Testing the ability of the compound to kill the cells by taking the advantage of various properties of cell

- Able to process the large number of compounds quickly with minimum of quantity.
- Range of concentrations used is comparable to that expected for in vivo studies.

DISADVANTAGES

- Difficulty in maintaining the culture
- Show negative results for the compounds which gets activated after metabolism and vice versa
- Impossible to ascertain the pharmacokinetics.

How to culture cell line

- Tumor cell line derived from several cancer types.
- Adaptable to a suitable growth medium.
- Show reproducible profile for growth and drug sensitivity.
- The lines were prepared and preserved using regents such as DMSO during freezing.
- Thawing- bringing the freezed ampoule to room temperature by slow agitation.

Cell lines for cancer

There are plenty of cell lines are available for research purpose. Only very few are listed [95].

Table 7. Cancer cell lines

S.no	Cell name	Tissue	Species
1	UM-UC	Bladder	Human
2	FM3A	Breast	Mouse
3	C 170	Colon	Human
4	SHP 77	Lung	Human
5	RAG	Kidney	Mouse
6	HF 1	Liver	Rat
7	MEWO	Skin	Human
8	TT	Thyroid	Human
9	OV	Ovary	Human
10	C 6	Neural(Gluialtumor)	Rat

ASSAY [96]

For energy metabolism and autophagy

- FAD assay
- ATP assay
- Lysosome detection
- Mitochondrial membrane potential assay
- Reactive oxygen species test

For nuclear signaling, DNA damage and cell proliferation

- P^{53}assay
- Topoisomerase II assay
- P^{21}assay
- Cell proliferation assay
- Mdm2 assay

For inflammation, angiogenesis and metastasis

- Cytokine and chemokine assay
- STAT 1,2,3,6 assay
- COX-2 activity assay
- LDL uptake assay

For apoptosis, pyroptosis and necrosis

- Caspase 1 assay
- Bax assay
- Cytolysis assay

For cancer signaling pathway and phenotype

- ERK assay
- c- AMP assay
- c- Jun test

IN VIVO MODELS:

Many animal species develop cancers spontaneously and are valuable for understanding the biology of sporadic cancer development in humans. The major use

of spontaneous cancer models is to compare the biology with human, in these animals are increasingly valuable for cross- comparison of response or resistance to clinical agents used for patients [97].

Animal models

1. Mouse cancer models
    a. GEM – Genetically Engineered Mouse Models
        a. Inbred mice (systematic sibling mating)
        b. Transplantation models

- Allograft models (syngeneic tumor tissuse derived from same genetic mouse)
- Xenograft models (actual human cancer cells or solid tumors are transplanted into host mouse)

- Carcinogen induced and spontaneous models
- Digestive system cancer induced by polycyclic aromatic hydrocarbons
- Chemically cancer induced by Cadmium and Arsenic.
- Radiation-skin cancer by ultraviolet radiation; leukemic changes by ionizing radiation.

I. Rat cancer models
    a. Genetically altered rats
        i) Treat embryos with DNA damage causing chemical mutagen. Frequently N-Ethyl-N-nitrosourea(ENU) is used.
        ii) Insertion of mutagenesis strategies (Retro viruses)
        iii) Transgenic strategies (pronuclear injection of DNA)- quickly developed and more effective models
    b. Inbred rats.

II. Other laboratory animal models
    a. Hamster
    b. Rabbits
    c. Zebrafish

III. Other animal models
    a. Dogs

b. Cats
c. Goats
d. Horses
e. Pigs

There is also work done with various species, such as baboons, chimpanzees, macaques, marmosets and tamarins.

Oral cancer cell lines

- Hep 2 (Human epithelial type 2 cells)
- FaDu
- SCC-4
- OEC-M1
- KB

Cerviacl cancer cell lines

- HeLa (HPV 16)
- SiHa (HPV 18)
- C 33A (HPV Negative)
- CaSki

Induction of cervical cancer in animal models:

- Cervical neoplasia is induced in mouse by an extract of varicella zoster virus infected cells (HPV or Herpes simplex virus type 2 DNA)
- Genomic HSV 2 DNA was isolated from infected HE p^2 cells and separated from host cell DNA by Cesium chloride density gradient centrifugation.
- The DNA was applied to mouse cervix for period of 80 to 100 weeks. Should be examined monthly to detect abnormalities.

3.4. PHARMACEUTICAL REVIEW:

PADHANGAM:

Concept and Terminology:

"*Padhangam"* is a sublimate. A number of organic and inorganic materials are capable of sublimation. Thus mercury as an element and red sulfide of mercury etc. sublime as known to us are from the medicinal drugs of inorganic nature. Camphor is

an organic substance that sublimes. Similarly, several other constituents of vegetable drugs sublimate [93].

In the preparation of the medicines sulfur, mercury, cinnabar, corrosive sublimate, calomel and white arsenic are inorganic substances that are sublimated and hence the suffix Padhangam to these medicines.

Method of preparation:

Sublimation process:

- The process of preparation involves the heating of the sublimating constituents in a suitable set up which is described under the individual preparations.
- In the conventional set up of the sublimation contrivance, a heat resistant pot is used as the container for the drug ingredients. When using pots, two identical pots of appropriate dimensions and capacity should be selected and checked for neat contact of rims when juxtaposed.
- The ingredients from which the sublime materials to be prepared are placed in the small pot. The juice of the leaf which will attract the deposition of the sublimated product is applied on the inner surface of the big pot.
- The big pot is placed over the small one in such a way that their mouths oppose each other. The gap between the rims is covered by seven layers of clay smeared cloth and is allowed to dry.
- This setup is placed on the oven and heat is applied, by burning fire wood. In the application of heat, there gradations are recognized.
- These three stages, mild, moderate and intense are best understood and mastered with some experience.
- It is said that, if the flames are convergent and resemble a single tongue of flame as in a lamp, it is mild fire (*Deepakkini*). If several such tongues of flame lick the vessel and diverge like the flower of lotus, it is moderate (*Kamalakkini*).

- If the multiple tongues of flame fill the oven and enrich the sand bath. It is the intense stage of fire (*Katakkini*).
- These stages of fire should be manipulated and followed as prescribed in the method of preparation.
- The heating is maintained for 12 hours.When the setup has cooled down, the final product is taken out and the clay tape winding cut out.
- The material that has sublimed in upper bowl is gently tapped with suitable beater or lifted with a spatula. The sublimate collected should be finely ground in a mortar.

Leaves used for applying the juices on the inner surface of the pot favouring deposition:

- Leaves of Datura metal (oomathai elai)
- Leaves of Erythrina veriegata (kalyana murungai elai)
- Piper beetle (vetrilai)
- Leaves of Coccinia grandis (kovai elai)
- Leaves of Ocimum sanctum (thulasi elai)
- Leaves of Acalypha indica (kuppaimeni elai) [98]

SHELF LIFE

The Padhangam are said to retain their potency for 10 years.

PRESERVATION AND STORAGE

To be stored in a clean, dry and air tight glass containers.

SPECIAL EXPERIMENT

- In a glass bowel containing water, sprinkle pinch of Padhangam and place a whole black gram on it.
- If Padhangam bears the entire weight of grain and doesn't sink in the water, the preparation method was perfect.

Table 8. ANALYTICAL SPECIFICATIONS OF PADHANGAM (RASAYOGA) [99]

S. NO	TEST
1	Description Colour Odour
2	Identification – chemical
3	Particle size – 200 to 300
4	Loss on drying 105^0 C
5	Total ash
6	Acid – insoluble ash
7	Water soluble ash
8	Assay of element
9	Ayurvedic specifications
10	Lusterless (Nishchandrica)
11	Fine enough to enter the crevices of finger (Rekha purnatva)
12	Floats on water (Varitara)
13	Smokeless (Nirdhoom)
14	Tasteless (Niswadu)
15	Irreversible (Apunarbhav)

3.5. LATERAL REVIEW

Pistia stratiotes

1. Methanalic extract of *Pistia stratiotes* leaves showed antimicrobial activity against both gram positive and gram negative Bacteria. highest zone of inhibition was observed at 1000mg/ml concentration against klebsiella species [100].

2. Methanalic extract of *pistia stratiotes* has shown highest antioxidant property, the reducing power of methanol extract indicates presence of some compounds in *pistia stratiotes* extracts which can donate electron and could react with free radicals to convert them into more stable products and to terminate radical chain reactions. increased absorbance of reaction mixture indicates increase reducing power of extract [101

3. *Pistia stratiotes* leaves possess antifungal properties the use of plant in folk medicines for the treatment of various diseases [102].

4. Methanalic extract of whole plant is able to lower the level of thyroid hormones. May regulate the hyperthyroidism [103]
5. *Pistia stratiotes*, shows with 30 days' the digestion time at temperature of 29.5,33.0 and 37.5 c respectively. The average methane content was 58-68%.is very suitable for biogas production [104]

6. The plant extract of *Pistia stratiotes* exhibited the anthelmintic activity in dose dependent manner giving shortest time of paralysis and death with 50 mg/ml concentration [105]
7. The Methanalic extract of *Pistia stratiotes* was tested by acetic acid –induced writhing model in mice at dose of 250 and 500 mg kg of body weight, the extract produced about 35.80 and 47.94% writhing inhibition in test animals. [106]
8. *Pistia stratiotes* showed significant anti diarrhoeal activity against castor oil-induced diarrhoea as compared with the control dosage 250 and 500 mg kg body weight. [106]

Oxalis cornuculata

1. *Oxalis cornuculata* Linn. showed the significant antifungal activity against *A. niger* by suppressing the fungal mycelial growth by 71 to 86% for after three days of incubation [107].

2. Methanol extracts of whole plant of *Oxalis cornuculata* using *Eisenia foetida* at three different concentrations (100, 200 and 400 mg/ml) the time of paralysis and death time was observed as 11.33 and 41.33, respectively [108].

3. Ethanolic extract of *Oxalis corniculata* having nematotoxic activity against phytoparasitic nematodes. In another research has revealed the ethanoic extract of *Oxalis cornuculata* after 7 days of incubation period the immobility of the nematode was observed under the light microscope and that confirm the nematocidal activity of this plant [109].

4. The alcoholic and petroleum ether extract of whole plant of *Oxalis corniculata* has been evaluated for its wound healing activity at the dose of 300 and 500 mg per kg showed significant wound healing activity by producing an increase in wound contraction rate, wound breaking and significant decreases in epithelization period [110].

5. Petroleum ether and ethanol extracts of the whole plant of *Oxalis corniculata* L was administered orally at the dose level of 100 and 200 mg/kg body weight from day 1 to 7 of pregnancy to evaluate the anti-implantation activity. showed significant anti-implantation activity when laparotomised on day 10, it was maximum of (76.42%). The pregnant rats which received the treatment from day 8 to 14 of pregnancy showed abortifacient activity and it was maximum of (78.55%) with high dose of petroleum ether extract [111]

6. Methanol extract of *Oxalis corniculata* Linn. (whole plant) antiulcer potential at the dose levels of 125, 250 and 500 mg/kg. It possesses significant antisecretory and antiulcer effects and justify the traditional usage of this herb to treat peptic ulcer [112]
7. The aqueous extract of the *Oxalis cornuculata* has been tested for the inhibitory potential against procaine pancreatic amylase. At a concentration of 100µg/ml exhibited a maximum inhibition of 89.27% (IC 50 value 68.08±0.06) oxalis

cornuculata, are a good source for controlling postprandial hyperglycemia, a major problem in type-II diabetes [113]

8. *Oxalis corniculata* identified several compounds that showed anti-amoebic activity in axenic cultures of *E. histolytica*. These were characterized by nuclear magnetic resonance, infrared and mass spectrometry as (i) Oc-1, a mixture of saturated fatty acids C24 to C28; (ii) Oc-2, a mixture of long-chain alcohols C18 to C28; and (iii) Oc-3, a single compound that was a galacto-glycerolipid (GGL). Of the different compounds that were obtained, the strongest anti-amoebic activity was found in GGL [114].

9. Ethanolic extract of *Oxalis corniculata* Linn. at doses of 200 and 400mg/kg body weight evaluated for its anti-nociceptive activity in diabetic neuropathy rats. Diabetic rats were showed significant reduction in tail flick latency by 49% in hot water tail immersion test and decreased paw withdrawal by 40% in hot plate test by the end of 5th week [115].

10. The anti-diarrhoeal activity of aqueous and methanolic extracts of *Oxalis corniculata* Linn. was evaluated on castor oil induced diarrhoea in rats and on small muscle intestinal transit at orally administered doses of 160,320 and 640 mg/kg of body weight. [116]

11. Methanolic extract of *Oxalis corniculata* leaves at doses of 200 and 400mg/kg body weight were screened for antiepileptic activity. In MES model, MEOC showed significant reduction in duration of hind leg extension with 200 mg/kg dose and effect was dramatically reduced with 400mg/kg. [117]

12. The hepatoprotective activity of ethanolic leaves extracts of *Oxalis corniculata* (200 and 400 mg/kg) were evaluated against thioacetamide-induced hepatotoxicity. Oral administration of *Oxalis corniculata* aqueous and ethanolic leaves extract at 400 mg/kg resulted in a significant reduction in SGOT (146.42±2.54 and 136.75±1.37 IU/L respectively), SGPT (81.96±3.15 and 72.05±2.33 IU/L respectively), GGTP (16.6±0.49 and 15.02±0.68 IU/L respectively), ALP (241.86±3.94 and 202.42±5.37 IU/L respectively) and total bilirubin (0.226±0.00 mg/dL 0.288±0.01 mg/dL respectively. [118]

13. The hypolipidemic and antioxidant activities of leaves of *Oxalis corniculata* Linn. extracts showed a significant decrease in total cholesterol, triglycerides, LDL and MDA in blood. On the other hand, HDL, CAT and SOD were increased significantly [119].

14. *Oxalis corniculata* Linn. has steroidogenic activity and this natural chemical can be safely used as it does not alter the functioning of organs which is proved by its action on one of the endocrine organ-adrenal gland, which functions normally in the extract treated female albino rats [120]

15. Antitumor activity of ethanolic extract of *Oxalis corniculata* against Ehrlich ascites carcinoma on Swiss mice was carried out at the doses of 100 and 400 mg/kg increased significantly ($p<0.05$ and $p<0.01$) [121]

16. The hypolipidemic and antioxidant activities of leaves of *Oxalis corniculata* extract was given at a dose of 500 mg/kg showed a significant decrease in total cholesterol, triglycerides, low density lipoprotein (LDL) and Malondialdehyde (MDA)in blood. On the other hand, high density lipoprotein (HDL), catalase (CAT) and Superoxide dismutase (SOD) were increased significantly [122].

Trianthema decandra

1. Leaf extract of *Trianthema decandra* exhibit central analgesic properties, since it exerted a significant and dose dependent effect on chemical (acetic acid and thermic heat) painful stimuli from the respective doses of 100 and 200 mg/kg such an efficacy on these two stimuli is characteristic of central analgesic like morphine, while peripheral analgesic (paracetamol and aspirin) and known to be inactive on thermic painful stimuli. [123]

2. The anti-inflammatory properties of chloroform extract of *Trianthema decandra* showed maximum inhibition of 58.36% at the dose of 200 mg/kg after 3 hrs of drug treatment in carrageenan induced paw oedema. [123]

3. The antimicrobial properties of chloroform extract of *Trianthema decandra* were studied against Gram positive, Gram negative bacteria and fungi by disc diffusion assay. Wound healing properties were determined using the excision wound model. [124]

4. Plant extracts on α-amylase inhibition assay (non-pre-incubation method) was adopted for evolution of anti-diabetic activity of *Trianthema decandra* Chloroform extract was found to have most inhibitory effect on α-amylase with 60 % inhibition at 3 min. Ethyl acetate extract was found to have inhibitory effect on α-amylase with 47.48 % inhibition at 3 min. [125]

5. The hepatoprotective activity of roots of *Trianthema decandra* aqueous extracts was evaluated against carbon tetrachloride induced hepatotoxicity the roots aqueous extract at the doses 100 ,200mg/kg has controlled the liver damage caused by carbon tetrachloride. [126]

6. Leaf extract of *Trianthema decandra.* essential oil showed the Diameter of Inhibition Zone (DIZ) ranging from 19 ± 0.01 to 24 ± 0.05 mm at a concentration level of 1 mg/disc in all the twelve strains tested. The minimal inhibitory concentration (MIC) of essential oil against bacterial and fungal strains was in the range of 625–1250 μg/ml [127].

7. *Trianthema decandra* showed Aphrodisiac activity in a dose dependent manner. The medium and high dose treated groups showed significant increase in the number of mounts, thrusting and decrease in the latency.The Aphrodisiac activity of aerial parts of *Trianthema decandra* and may be attributed to elevation of Testosterone, Adrenergic, Cholinergic & Dopamine levels [128]

8. Alcoholic extract of *Trianthema decandra* Linn., at various doses using experimental induced stress models in Mice and Rats. The phyto fragments

found during GC-MS analysis might also contributed to the Adaptogenic activity of alcoholic extract of *Trianthema decandra* [129]

Hydragyrum perchloride

1. *VRP and PMC* drugs, inhibited proliferation of MCF-7 breast cancer cells in time and dose dependent manner. Concentration *VRP and PMC* drugs increased from 10 – 50 μg/mL, and the percentage of inhibition increased from 63.62% to 90.16% for VPR at 24 to 48 h and 65.03 % to 70.51 % for PMC at 24 to 48 h of these two drugs subjected to the test, VPR was more efficacious since it affected viability of the cells its statistically significant at the level of $P < 0.05$. [130]

2. Amongst various concentration of drug(VM) tested 500 UG/ml showed maximum absorbance of 0.53 0.01. The *Veera Mezhugu* possess both antioxidant anti-cancer potentials justifying scientifically administration in cancer. [131]

3. *The Kodasuri veeravaippu* possess inhibition of the synthesis of arachidonic acid metabolites via inhibiting COX-2. Kodasuri veeravaippu possess significant anti-inflammatory activity on both acute and chroic inflammation. [132]

4. "*Kodasuri veeravaippu*" showed good inhibitory activity on almost all the bacteria used. Gram positive bacterial strain, Staphylococcus aureus and the gram negative strain Escherichia coli were found to be more susceptible to the test drug "*Kodasuri veeravaippu*" by showing inhibition zone of 30mm and 22mm respectively. [133]

5. *The Aya*veera chendooram is good anti vatha effect treating for vathasthambam.at the dosage 130mg twice a day with honey.[134]

6. Anti-atherosclerotic activity of *Pavala chendhooram* the dosage of 50mg for a period of 6months.

7. Anna *Pavala Chendhooram* reduced the plasma cholesterol reduced up to 65% and the HDL level was increased. The atheroma formation was also inhibited sphingomyelin levels. [135]
8. Hemostatic activity of *Pavala parpam* treated with dose 500mg/kg body weight. The blood showed marked reduction in both bleeding and clotting time. [135]

9. Hepato-protective activity of *Kodi pavala chunnam* for its hepatoprotective activity in experimental rats. Liver damage was induced by CCl4 in Wister rats. The liver damage was assessed by hematological and biochemical parameters. Showed near normal levels in hematological, biochemical parameters which indicate the hepato-protective activity. [135]

10. The drug *Pavala parpam* was evaluated for ant osteoporotic activity in experimental rats. The drug treated group received *Pavala parpam* 65 mg/kg body weight, twice a day for 16 weeks. The decreased femoral weight and density were significantly reversed in animals treated with *Pavala parpam*. The cortical bone morphometric indices also revealed raised medullary. width and cross-sectional area in treatment group. The combined cortical thickness and cortical and periosteal area ratio are also increased compared to sham operated animals. [135]

11. *Amirtha vennai* (external application) used for all skin disease. [136]

12. *Kanakalinga karpura mezhugu*. Mezhugu was investigate with scientific parameters to reveal many ascertained drugs for present day refractory diseases like cancer, rheumatoid arthritis, thyroid disorders, bronchitis, benign growths, adenitis and swellings. [137]

4. MATERIALS AND METHODS

SELECTION OF THE DRUG:

For this present study, the Herbo-mineral formulation *"**BHRAMASTHIRAM**"* was taken as the compound drug preparation for cancer mentioned in the classical Siddha literature "**The pharmacopeia of Siddha Research Medicines"** written by **Dr.M.Shanmugavelu, Dr.G.D.Naidu L.I.M.,H.P.I.M.** published by G.D. Naidu president, I.L.W.A. ltd, printed at I LWA press,Coimbatore-18.[138]

Table 9. MINERALS INGREDIENTS

S.NO	NAME OF DRUGS	CHEMICAL NAME	QUANTITY
1	*Veeram*	Hydragyrum Perchloride	70gm(6 Thola)
2	*Kariyuppu*	Sodium chloride	3kg(6 lbs)

Table 10. HERBAL INGREDIENTS

S.NO	DRUG NAME	BOTANICAL NAME	QUANTITY
1	Vellai Saranai	Trianthema decandra	30kg
2	Puliyarai	Oxalis cornuculata	2kg
3	Aagayathamarai	Pistia stratiotes	6kg

Collection of the raw materials:

- All the raw materials were purchased from R.N.Rajan country drug store, Parrys corner, Chennai.
- Fresh plant material *Agayathamarai* were collected from, Thirumangalam Village, Kancheepuram dist.
- *Pulliyara*i were collected from kolli hills.
- *Vellai saranai* collected from Vanthavasi Hills, and ABS garden Salem.

Identification and Authentication of the drug:

The raw materials were identified and authenticated by the *experts* of *Gunapadam*, Government Siddha Medical College, Arumbakkam, Chennai- 106. The specimen sample of each raw material has been kept in the PG *Gunapadam* department individually for future reference.

Purification of the drugs

Purification process was done as per classical Siddha literature [16]

4.1. Purification of Raw drugs

1. Purification of *Veeram* (Hydragyrum perchloride)

Materials Required:

- Camphor
- Tender coconut water
- Perchloride of mercury

Procedure:

Camphor is mixed with tender coconut water and placed in a mud pot. Per chloride of mercury is tied in a cloth and soaked in the pot without touching the water and the pot is burnt for half an hour.

2. Purification of *Kariyuppu (Sodium Chloride)*

- Common salt -500gm
- Sea water -1 lit

Procedure:

Common salt is dissolved in sea water and filtered. The filtrate is boiled till it reaches semi consistency state. It is dried in day light and it attains the solid state as purified salt.

3. Purification of *Aagayathamarai* (Pistia stratiotes)

Removed the olden leaves and washed with water.

4. Purification of *Vellai Saranai* Leaves (*Trianthema decandra*)

Removed the olden leaves and washed with water.

5. Purification of *Puliyarai* Leaves (*Oxalis Cornuculata*)

Removed the olden leaves and washed with water.

4.1.2. Preparation of the trial drug – *Bhramasthiram* [138]

Minerals materials:

1. *Veeram* (Hydragyrum Perchloride) - 70gm

2. *Kariyuppu* (Sodium chloride) - Sufficient quantity(3.kg)

Plant materials:

3.Vellai Saranai(*Trianthema decandra*) - Sufficient quantity(25 kg)

4. Puliyarai (*Oxalis coriculata*) - Sufficient quantity (1 kg)

5. Aagayathamarai (*Pistia stratiotes*) - Sufficient quantity (6 kg)

PROCEDURE:

- A mud pot was taken. Into which *Vellai saradai* (T*rianthema decandra*) leaves are spread into a thin layer.
- Then 1/8 part of the salt was spread over the leaves, by layers.
- This process was repeated alternatively with the same quantity of leaves and salt.
- The mud pot was filled with three layer of leaves and 3/8part of salt.
- Similarly, the rest of the leaves and salts were spread one over the other so as to be arranged in eight such layers. The top most layer should be the leaves.
- The mud pot was covered with a lid and then sealed with the seven layers of mud pasted cloth and let dried.
- It was subjected to calcination using cow dung cakes which is about 8-10 times the weight of the *kavasam*.
- It is then cooled and the product (salt) was collected carefully.

- The process of ignition was repeated for about ten times, fresh leaves of *Vellai saradai* is to be used each time.
- Then the product of ignition was divided into 10 equal parts. The first part was placed in the *kalvam* and rubbed with the juice of *puliyarai* leaves and made into a paste.
- The above paste used as *kavasam* for sealing the *savveram* mass and dried in the hot sun.
- The above process was repeated with the remaining 9 parts and dried for 10 days.
- Then, the *veera kavasam* was placed in an *Erippu chutty* (earthenware) and is covered with a suitable lid and then subjected to *pudam* (calcination).
- It was ignited with low flame *Deepakini & Kamalakini* each for about 1 *Saamam* (3 hours).
- Then it was followed by iginition with high flame(*kadaagini*) for about 6 hours.
- It was then allowed to cool. After that the pot was unsealed and *pathangam* was collected carefully.
- Finally, the mixture was collected, weighed and kept in an air tight container and was labeled as *BA*.

Dosage	**:**	1/2-1 grains *(32.5-65 mg)*
Form of Medicine	**:**	*Padhangam* (sublimation)
Route	**:**	Enteral (oral)
Time of Administration	**:**	Two times a day in empty stomach.
Adjuvant	**:**	*Jaggery*
Indications	**:**	P*utru* (cancer), leprosy, skin disease, *Vadha* Disease.

Analysis as per AYUSH guidelines:

1.Floating on Water:

A pinch of BA gently placed on the still surface of water in a vessel, did not sink immediately. It was found that the *Padhangam* particles floated over the surface of water indicated lightness of the trial drug.

2. Lines on fingers:

Padhangam in well prepared form should be fine. When taken between thumb and index finger, the fine powder will fill up the lines of the finger print.

A pinch of *BA* was taken in between the thumb and index finger and rubbed. It was found that the *BA* entered into the lines of the finger, and was not easily washed out from the lines, confirmed its fineness.

3. Irreversible reaction:

The well prepared *Padhangam* does not reversible to its metallic state when heated with a mixture of cane jaggery, hemp powder, ghee and honey. A pinch of *BA* was taken and mixed with cane jaggery, ghee and honey. It was observed that the *BA* did not reversible to its metallic state.

4. Tasteless:

The well prepared *Padhangam* should be completely tasteless. Presence of any taste like sweet or bitter indicates incomplete preparation which needed another calcination process. When a small amount of *BA* was kept on the tip of the tongue, no specific taste was found

5. Lusterless:

If any shining particles present in *Padhangam*, it indicates that the *Padhangam* was not manufactured properly and contains unchanged substances like minerals, metals and other toxic substances. There should be no shining particles present in the well manufactured *Padhangam*. The *BA* was taken in a Petri bowl and observed for any luster in daylight through magnifying glass. No luster was observed in the *Padhangam*.

4.2. DRUG STANDARDIZATION:

Standardization of drug means confirmation of its identity, determination of its quality, purity and detection of nature of adulterant by various parameters like morphological, microscopically, physical, chemical and biological evaluations [140]

STANDARDIZATION OF THE DRUG *BA*:

Standardization of drugs helps to prove its identity and determination of its quality and potency. Standardization of the Herbo- mineral formulation was based on the qualitative and quantitative analysis through physico-chemical investigations and instrumental analysis. The physico-chemical analysis of the prepared Herbo- Mineral drug have been done at The Tamilnadu Dr M.G.R Medical University, Anna salai, Guindy, and elemental analysis have been done at Tamilnadu test House, Vanagaram, Chennai 95. (FTIR, SEM, ICP-MS, XRD).

4.2.1. ORGANOLEPTIC CHARACTER

The organoleptic characters of the sample drug were evaluated as per the standard procedure7.1gm of the *BA* was taken and the colour, texture, particle size and other morphology were viewed by naked eye under sunlight. Then the result was noted.

4.2.2. PHYSICO-CHEMICAL ANALYSIS:

Physico-chemical investigations like pH value, Loss on drying at 105°C, solubility test, Determination of total ash, Determination of acid insoluble ash, Determination of water soluble ash, Determination of water soluble extractive, Determination of alcohol soluble extractive, have been done at The Tamilnadu Dr M.G.R Medical University, Anna salai, Guindy, as per the guide lines of WHO [141].

pH value:

Potentiometrically, pH value is determined by a glass electrode and a suitable pH meter. The pH of the *BA* was written in results column.

Loss on Drying:

An accurately weighed 2gram of Bhramasthiram formulation was taken in a tarred glass bottle. The crude drug was heated 105^0 c for 6 hours in an oven till a

constant weight. The percentage moisture content of the sample was calculated with reference to the shade dried material.

Solubility test:

A pinch of sample[*BA*] was taken in a dry test tube and it 2ml of the solvent was added and shaken well for about a minute and the result are observed. the test was done for solvent like distilled water, Ethanol, petroleum, ether, propylene glycol, Toluene, Benzene, chloroform, Ethyl alcohol, Xylene, Carbon tetra chloride, and the result are observed individually.

Determination of total Ash:

Weighed accurately 2gram of Bhramasthiram formulation was added in crucible at a temperature 600^0c in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air dried drug.

Determination of acid insoluble ash:

Ash above obtained was boiled 5min with 25ml of hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble as was calculated with reference to the air dried drug.

Determination of water soluble ash:

Toal Ash 1gram was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with water and ignited for 15 min at a temperature not exceeding 450^0c in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

Determination of water soluble extractive:

5 gram of air dried drug. Coarsely powered Bhramasthiram was macerated with 100ml of distilled water in a closed flask for twenty-four hours, shaking frequently. The solution was filtered and 25 ml of filtered was evaporated in a tarred flat bottom shallow dish, further dried at 1000c and weighted. The percentage of water soluble extractive was calculated with reference to the air dried drugs.

Determination of alcohol soluble extractive:

2.5gram of air dried drugs coarsely powdered Bhramasthiram was macerated with 50ml. alcohol in closed flask for 24 hours. With frequent shaking.it was filtered rapidly talking precaution against loss of alcohol .10ml of filtrate was the evaporated in a tarred flat bottom shallow dish, dried at 1000 c and weighed. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.

4.2.3. BIO-CHEMICAL ANALYSIS

The bio-chemical analysis was done to identify the acid and basic radicals present in the *BA*.

Methodology of chemical analysis:

Preparation of extract: 5gram of *BA* was taken in a 250 ml clean beaker and 50 ml of distilled water was added, boiled well and allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water.

4.2.4 PRELIMINARY BASIC AND ACIDIC RADICAL STUDIES[142]

Table 11. **Preliminary Basic and Acidic Radical Studies**

S.no	PROCEDURE	OBSERVATION	INFERENCE
1	**Test for potassium**: A pinch of *BA* 2ml of sodium nitrate and 2ml of cobalt nitrate solution in 30% glacial acetic was added	Formation of yellow colored precipitate	Presence of potassium
2	**Test for calcium:** To 2ml of extract in a clean test tube. Then acetic acid and potassium chromate solution were added.	Formation of yellow precipitate	Presence of calcium.
4.	**Test for Ammonium:** 2ml of extract was taken in a test tube and add few ml of Nessler's reagent	Appearance of brown colour	Presence of Ammonium

5.	**Test for Sodium:** 2 pinches of BA were mixed with HCl and made into paste. And introduced into the blue flame of Bunsen burner	Appearance of intense yellow colour	Presence of Sodium
6.	**Test for Iron (Ferrous):** 2ml of extract was taken in a clean dried test tube and conc. HNO_3 and ammonium thiocyanate were added.	Appearance of blood red colour	Presence of Iron
7.	**Test for Zinc:** 2ml of extract was taken in a test tube and potassium Ferro cyanide solution was added.	Formation of white colour precipitate	Presence of Zinc
8.	**Test for Aluminium:** 2ml of extract was taken in a test tube and sodium hydroxide was added to it.	Formation of White colour precipitate	Presence of Aluminium
9.	**Test for Lead:** 2ml of extract was taken in a test tube and add 2ml of potassium iodide solution	Formation of yellow colour precipitate	Presence of Lead
10.	**Test for Copper:** To a small portion of the extract dilute hydrochloric acid was added and then hydrogen sulfide gas is passed through the solution	Formation of black precipitate	Presence of Copper
11.	**Test for Mercury:** 2ml of the extract was taken in a test tube and treated with 2ml of sodium hydroxide solution	Formation of yellow precipitate	Presence of Mercury

12.	**Test for Arsenic:** 2ml of the extract was taken in a test tube and treated with 2ml of sodium hydroxide solution.	Formation of brownish precipitate	Presence of Arsenic

Results were noted and tabulated in Table no: 17

Table12. **Test for acid radicals**

S.no	PROCEDURE	OBSERVATION	INFERENCE
1	**Test for Sulphate:** 2ml of extract was taken in clean, dry test tube and 5% barium chloride solution was added to it	Formation of white precipitate	Presence of Sulphate
2	**Test for Chloride:** The extract was taken in a test tube and then treated with silver nitrate solution	Formation of white precipitate	Presence of Chloride
3	**Test for Phosphate:** The extract was taken in a test tube and treated with ammonium molybdate and conc. HNO_3	Formation of yellow precipitate	Presence of Phosphate
4	**Test for Carbonate:** The substance was taken in a clean dry test tube and then treated with conc.HCl	Formation of effervescence	Presence of Carbonate

5	**Test for fluoride & oxalate**: 2ml of the extract was taken in a test tube and add 2ml of dil. Acetic acid, 2ml calcium chloride solution and then heated	Formation of cloudy appearance	Presence of Fluoride & Oxalate
6	**Test for Nitrate:**1gm of *BA* was heated with copper turnings and conc.H_2S0_4 and observe the test tube vertically down	Characteristic changes	Presence of Nitrate

Results were noted and tabulated in Table no:18

4.2.5 ANTI-MICROBIAL ACTIVITY

AVAILABILITY OF BACTERIAL LOAD [143]:

Enumeration of bacteria by plate count – agar plating technique

The plate count technique is one of the most routinely used procedure because of the enumeration of viable cells by this method.

Principle:

This method is based on the principle that when material containing bacteria is cultured, every viable bacterium develops into a visible colony on a nutrient agar medium.

The number of colonies, therefore are the same as the number of organisms contained in the sample.

Dilution:

A small measured volume is mixed with a large volume of sterile water or saline called the diluent or dilution blank. Dilutions are usually made in multiples of ten. A single dilution is calculated as follows:

$$\text{Dilution} = \frac{\text{Volume of the sample}}{\text{Total volume of the sample and the diluent}}$$

Requirements:

- Sample or Bacterial suspension
- K9 ml dilution blanks (7)
- Sterile petri dishes (12)
- Sterile 1 ml pipettes (7)
- Nutrient agar medium (200 ml)
- Colony counter

Procedure:

1. Label the dilution blanks as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}.
2. Prepare the initial dilution by adding 1 ml of the sample into a 9 ml dilution blank labelled 10^{-1} thus diluting the original sample 10 times.
3. Mix the contents by rolling the tube back and forth between hands to obtain uniform distribution of organisms.
4. From the first dilution transfer 1 ml of the suspension while in motion, to the dilution blank 10^{-2} with a sterile and fresh 1 ml pipette diluting the original specimen to 100 times.
5. From the 10^{-2} suspension, transfer 1 ml of suspension to 10^{-3} dilution blank with a fresh sterile pipette, thus diluting the original sample to 1000 times.
6. Repeat this procedure till the original sample have been diluted 10,000,000 times using every time a fresh sterile pipette.
7. From the appropriate dilutions transfer 1ml of suspension while in motion, with the respective pipettes, to sterile petri dishes. Three petri dishes are to be used for each dilution.
8. Add approximately 15 ml of the nutrient medium, melted and cooled to 45^0c, to each petri dish containing the diluted sample. Mix the contents of each dish by rotating gently to distribute the cells throughout the medium.
9. Allow the plates to solidity.
10. Incubate these plates in an inverted position for 24-48 hours at 37^0c.

Observation:

Observe all the plates for the appearance of bacterial colonies. Count the number of colonies in the plates.

Calculate the number of bacteria per ml of the original suspension as follows:

$$\text{Organisms per millimetre} = \frac{\text{Number of colonies (average of 3 replates)}}{\text{Amount of plated} \times \text{dilution}}$$

Results were noted and tabulated in Table no: 16

4.2.6. SOPHISTICATED INSTRUMENTAL ANALYSIS

FT-IR (Fourier Transform Infra-Red)

DEFINITION:

FTIR offers quantitative and qualitative analysis for organic and inorganic samples. Fourier Transform Infrared Spectroscopy (FTIR) identifies chemical bonds in a molecule by producing an infrared absorption spectrum. The spectra produce a profile of the sample, a distinctive molecular fingerprint that can be used to screen and scan samples for many different components. FTIR is an effective analytical instrument for detecting functional groups.

APPLICATIONS:

- Quantative scans
- Qualitative scan solids, liquids, gasses
- Organic samples, inorganic samples
- Unknown identification
- Impurities screening
- Formulation
- Pharmaceuticals

INSTRUMENT DETAILS

Principle:

SOPHISTICATED INSTRUMENTAL ANALYSIS

FT IR - Fourier Transform Infra-Red Spectroscopy [144].

FTIR (Fourier Transform Infra-Red Spectroscopy) is a sensitive technique particularly for identifying organic chemicals in a whole range of applications although it can also characterize some inorganics.

Examples include paints, adhesives, resins, polymers, coatings and **drugs**. FTIR is an effective analytical instrument for detecting functional groups.

APPLICATIONS:

- Quantative scans
- Qualitative scan solids, liquids, gasses
- Organic samples, inorganic samples
- Unknown identification
- Impurities screening
- Formulation

Principle:

Spectrophotometric tests are commonly used in the Identification of chemical substances and quantification of polymorphic forms. The test procedures are applicable to substances that absorb IR radiation.

The IR absorption spectrum of a substance compared with that obtained concomitantly for the corresponding reference standard / reference substance provide conclusive evidence of the identity of the substance being tested.

Recording Infrared spectrum of a solid as a disc (as per USP ‹197K›):

- Triturate about 1 to 2 mg of the substance to be examined with 300 to 400 mg, unless otherwise specified, of finely powdered and dried potassium bromide. If the substance is a hydrochloride it is preferable to use potassium chloride.
- Carefully grind the mixture and spread it uniformly in a suitable die.

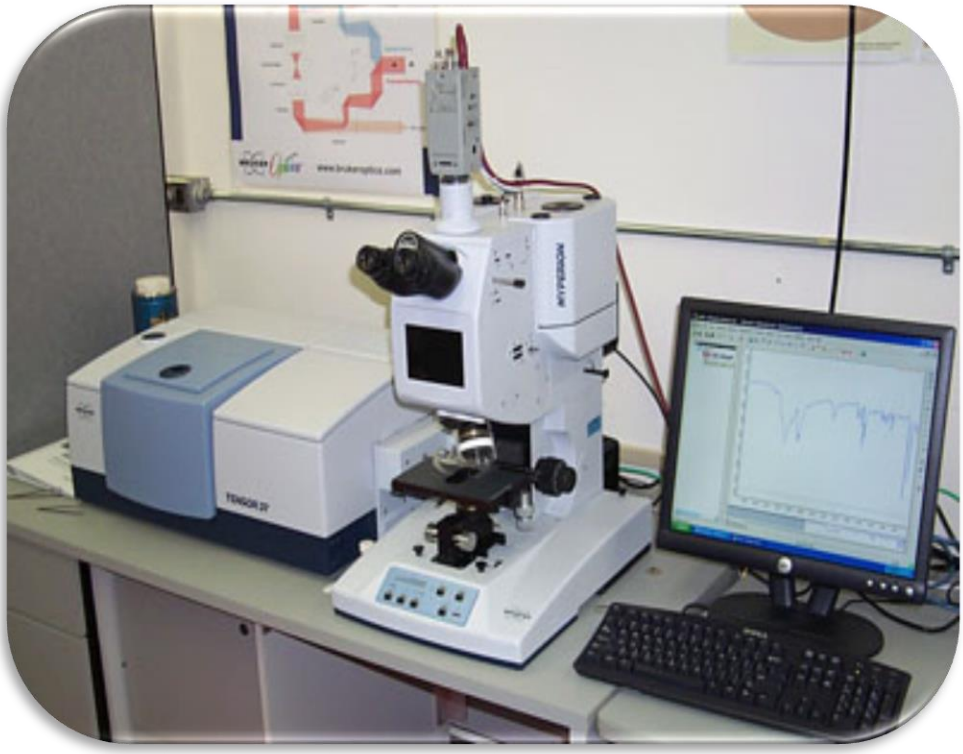


Figure12. FTIR INSTRUMENT

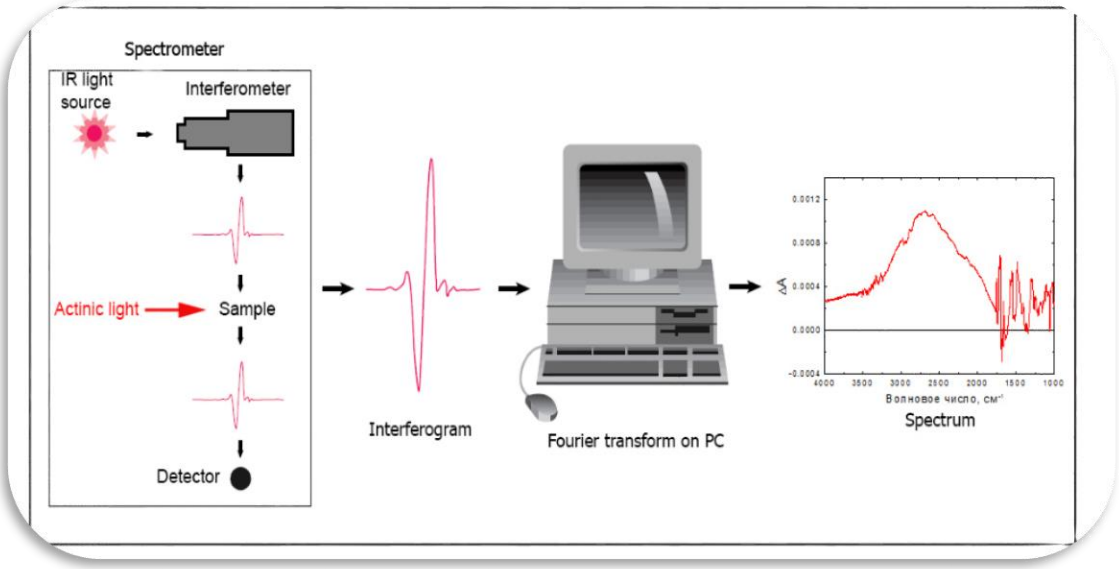


Figure 13. FTIR MECHANISM

- Submit it to the pressure of about 800 mPa (8 tons/cm2).
- Examine the disc visually and if any lack of uniform transparency is observed, reject the disc and prepare again.
- Record the spectrum between 4000 to 650 cm-1 unless otherwise specified in individual standard test procedure.
- When sample and standard are measured for concordance, the transmittance obtained at the start of the scan range, should not deviate by more than 10% between them (For eg. If the standard shows a transmittance of 75%, the sample transmittance can be between 65% and 85%).

FT-IR was the most advanced and the major advantage was its

- Speed
- Sensitivity
- Mechanical Simplicity
- Internally Calibrated

ICP-MS - INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

Analysis of Trace Metal and Inorganic Materials

Inductively Coupled Plasma Mass Spectrometry is a technique routinely used to analyse trace levels of a wide range of inorganic elements.

The ICP-MS allows for the detection and quantification of elements with atomic mass ranges 7 to 250. This covers Lithium to Uranium.

The typical detection limits are in the parts per billion (ppb) range and even parts per trillion (ppt) in some cases.

The ICP-MS analysis methods available at LPD Lab Services allow the detection, identification and quantification of a wide array of elements using a Perkin Elmer ELAN 6000 ICP-MS

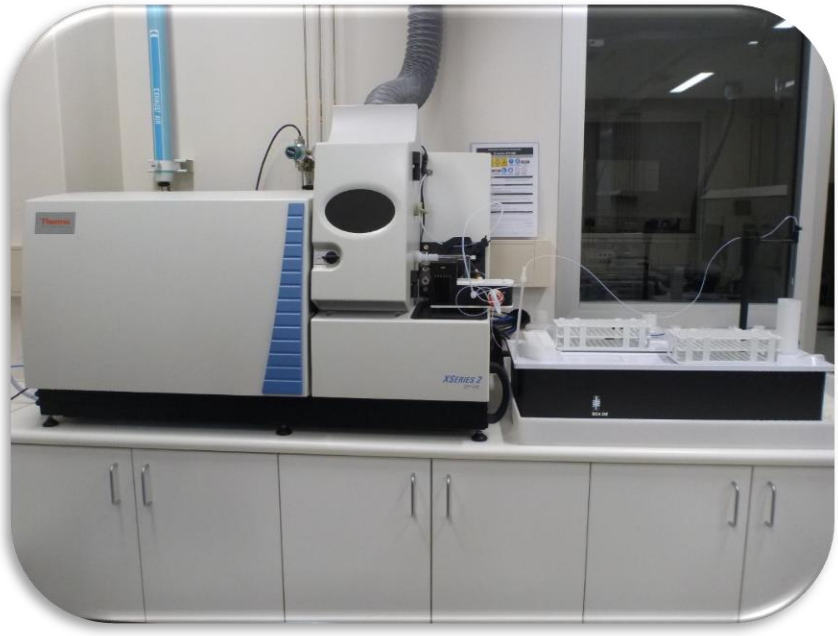


Figure 14. **ICPMS- INSTRUMENT**

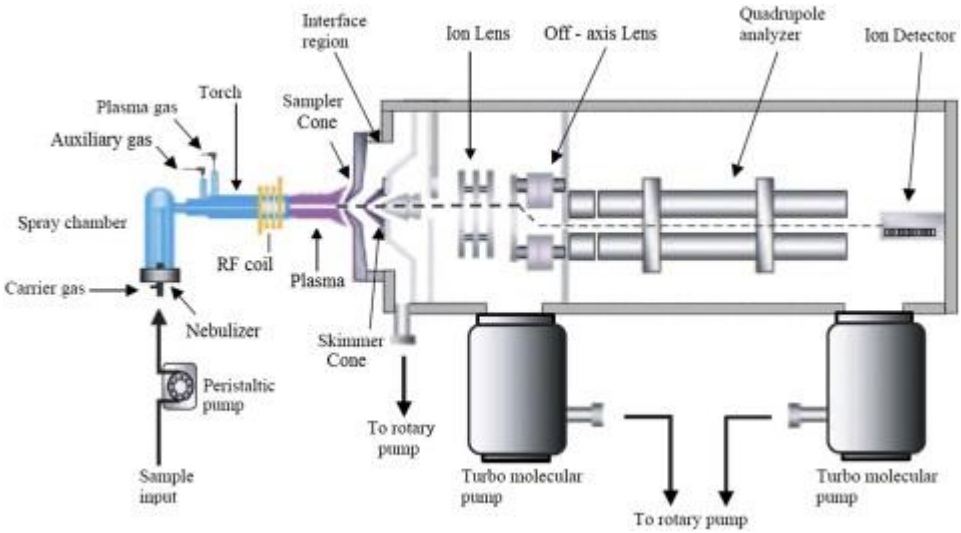


Figure 15. **ICP MS MECHANISM**

Analysis: Analyze according to the manufacturer's suggestions for program and m/z. Calculate and report results based on the original sample size.

Applications of ICP-MS

- Monitoring of trace metals in drinking water, ground water, rainwater, wastewater or industrial effluent streams.
- Trace elements in product / raw materials or from washed or rinsed surfaces.
- Analysis of additives and purity in metal alloys.
- Analysis of low level contaminants in chemical products, beverages, foods, cosmetics, pharmaceuticals.
- Analysis of soluble / leachable material from solid samples such as medical devices, polymers, PCB`s.
- Analysis can be performed on a diverse range of sample. [145]

SEM - Scanning Electron Microscope

DEFINITION:

Scanning Electron Microscopy (SEM), also known as SEM analysis or SEM microscopy, is used very effectively in microanalysis and failure analysis of solid inorganic materials. Scanning electron microscopy is performed at high magnifications, generates high-resolution images and precisely measures very small features and objects.

SEM ANALYSIS APPLICATIONS

The signals generated during SEM analysis produce a two-dimensional image and reveal information about the sample including:

External morphology (texture)

- Chemical composition (when used with EDS) Orientation of materials making up the sample
- The EDS component of the system is applied in conjunction with SEM analysis to:

- Determine elements in or on the surface of the sample for qualitative information.

- Measure elemental composition for semi-quantitative results.
- Identify foreign substances that are not organic in nature and coatings on meta.l
- SEM Analysis with EDS – qualitative and semi-quantitative results.
- Magnification – from 5x to 300,000x.
- Sample Size – up to 200 mm (7.87 in.) in diameter and 80 mm (3.14 in.) in height.
- Materials analyzed – solid inorganic materials including metals and minerals.
- Scanning Electron Microscopy uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens.

- In most SEM microscopy applications, data is collected over a selected area of the surface of the sample and a two-dimensional image is generated that displays spatial variations in properties including chemical characterization, texture and orientation of materials.

- The SEM is also capable of performing analyses of selected point locations on the sample. This approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions, crystalline structure and crystal orientations.

- The EDS detector separates the characteristic X-rays of different elements into an energy spectrum and EDS system software is used to analyses the energy spectrum in order to determine the abundance of specific elements.

- A typical EDS spectrum is portrayed as a plot of X-ray counts vs. energy (in Kev). Energy peaks correspond to the various elements in the sample.

THE SEM ANALYSIS PROCESS

Energy Dispersive X-ray Spectroscopy can be used to find the chemical composition of materials down to a spot size of a few microns and to create element

Figure16. **SEM INSTRUMENT**

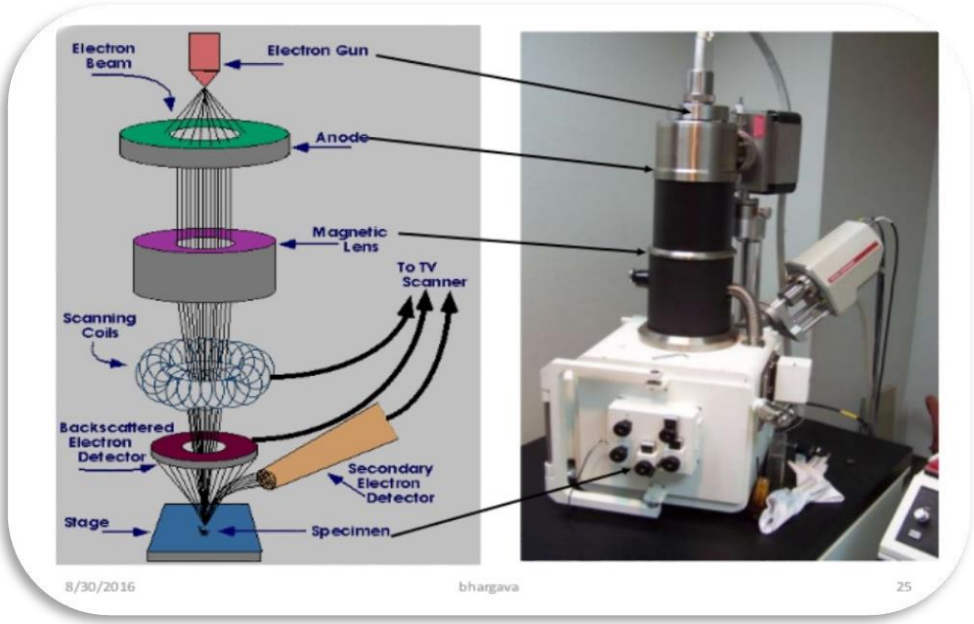


Figure 17.**SEM MECHANISM**

composition maps over a much broader raster area. Together, these capabilities provide fundamental compositional information for a wide variety of materials, including polymers.

In scanning electron microscope high-energy electron beam was focused through a probe towards *BA.* Variety of signals was produced on interaction with the

surface of the sample. This results in the emission of electrons or photons and it was collected by an appropriate detector.

The types of signal produced by a scanning electron microscope include:

- Secondary electrons
- back scattered electrons
- characteristic x-rays light
- specimen current
- Transmitted electrons.

This gives the information about the sample and it includes external morphology, texture, its crystalline structure, chemical composition and it displays the shape of the sample. [146]

XRD - X-ray Powder Diffraction (XRD

X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions.

The analyzed material is finely ground, homogenized, and average bulk composition is determined.

DEFINITION

X-ray powder diffraction is most widely used for the identification of unknown crystalline materials (e.g. minerals, inorganic compounds). Determination of unknown solids is important to studies in geology, environmental science, material science and biology.

APPLICATIONS:

- Characterization of crystalline materials
- Identification of fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically
- Determination of unit cell dimensions.

With specialized techniques, XRD can be used to:

- Determine crystal structures using Rietveld refinement
- Determine of modal amounts of minerals (quantitative analysis)
- Characterize thin films samples by:
- Determining lattice mismatch between film and substrate and to inferring stress and strain
- Determining dislocation density and quality of the film by rocking curve measurements
- Measuring super lattices in multilayered epitaxial structures
- Determining the thickness, roughness and density of the film using glancing incidence X-ray reflectivity measurements
- Make textural measurements, such as the orientation of grains, in a polycrystalline sample.

Strengths and Limitations of X-ray Powder Diffraction:

Strengths:

- Powerful and rapid (< 20 min) technique for identification of an unknown mineral in most cases, it provides an unambiguous mineral determination
- Minimal sample preparation is required
- XRD units are widely available
- Data interpretation is relatively straight forward.

Limitations:

- Homogeneous and single phase material is best for identification of unknown
- Must have access to a standard reference file of inorganic compounds

- Requires tenths of a gram of material which must be ground into a powder
- For mixed materials, detection limit is ~ 2% of sample for unit cell determinations, indexing of patterns for non-isometric crystal systems is complicated.

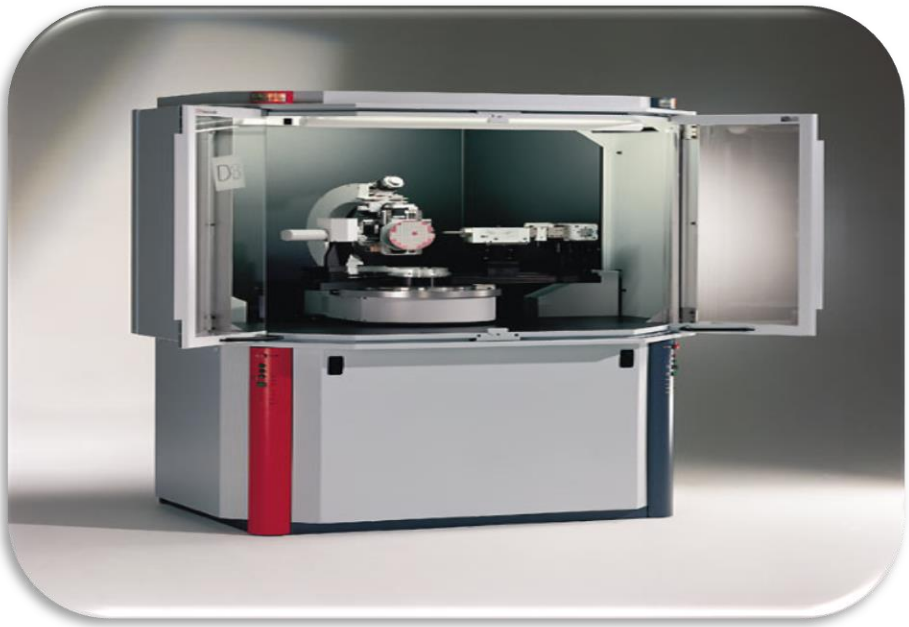


Figure 18.XRD - X-ray Powder Diffraction

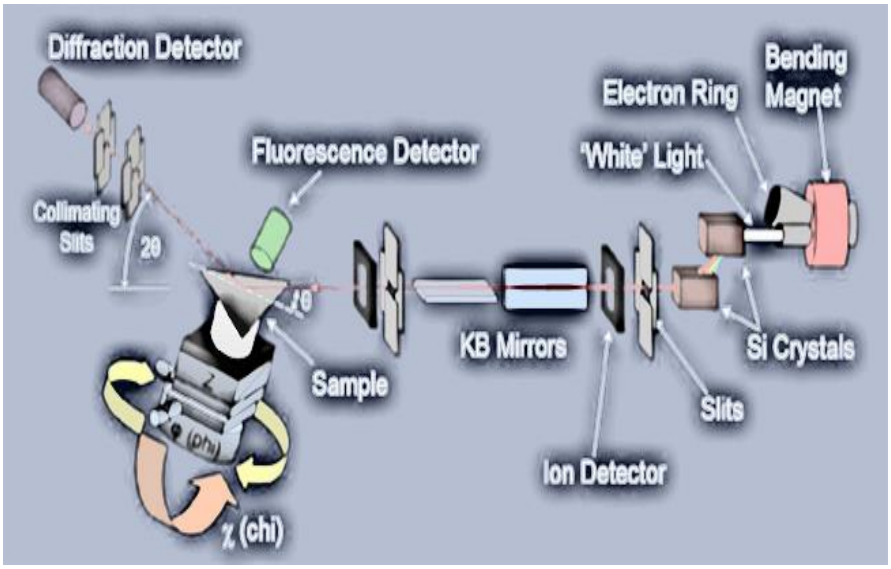


Figure 19. XRD Mechanism

Sample Collection and Preparation:

Determination of an unknown requires: the material, an instrument for grinding, and a sample holder.

- Obtain a few tenths of a gram (or more) of the material, as pure as possible
- Grind the sample to a fine powder, typically in a fluid to minimize inducing extra strain (surface energy) that can offset peak positions, and to randomize orientation. Powder less than ~10 μm (or 200-mesh) in size is preferred
- Place into a sample holder or onto the sample surface. [147]

4.3. TOXICOLOGICAL STUDIES

4.3.1.Acute oral toxicity

Acute oral toxicity study of *Bhramasthiram[BA]*-(OECD guideline – 423)

Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co - operation and Development, Guideline-423. [148]

IAEC No: IAEC/XLIV/20/CLBMCP/2016, C.L.BaidMetha College of Pharmacy, Thoraipakkam, Chennai.

Introduction:

- The acute toxic class method is a stepwise procedure with the use of 3 animals of a single sex per step.
- Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgement on the acute toxicity of the test substance.
- This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods.
- The acute toxic class method is based on biometric evaluations with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment.

- In principle, the method is not intended to allow the calculation of a precise LD50, but does allow for the determination of defined exposure ranges where lethality is expected since death of a proportion of the animals is still the major endpoint of this test.
- The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.
- The use of a selection of pre-defined doses, regardless of test substance, with classification explicitly tied to number of animals observed in different states improves the opportunity for laboratory to laboratory reporting consistency and repeatability.

Principle of the Test:

It was the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance was administered orally to a group of experimental animals at one of the defined doses.

The substance was tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.

- No further testing is needed
- Dosing of three additional animals, with the same dose
- Dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

Methodology:

Selection of Animal Species

The preferred rodent species was the wistar albino rat, although other rodent species may be used. Healthy young adult animals are commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each

animal, at the commencement of its dosing, should be between 6 to 8 weeks old and the weight (150-200gm) should fall in an interval within±20 % of the mean weight of any previously dosed animals.

Housing and Feeding Conditions

The temperature in the experimental animal room should be 22ºC ± 3ºC. Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hour's light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water.

Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Preparation of animals:

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions.

Test Animals and Test Conditions:

Sexually mature Female Wistar albino rats (150-200gm) were obtained from TANUVAS, Madhavaram, Chennai.

All the animals were kept under standard environmental condition (22±3°C). The animals had free access to water and standard pellet diet (Sai meera foods, Bangalore).

Preparation for Acute Toxicity Studies

Rats were deprived of food overnight (but not water 16-18 h) prior to administration of the, *BHRAMASTHIRAM* ***(BA)***.

The principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of the animals and the study design

IAEC approved Number	: IAEC/XLIV/20/CLBMCP/2016
Test Substance	: *BHRAMASTHIRAM* **(BA)**
Animal Source	: TANUVAS,Madhavaram, Chennai.
Animals	: Wister Albino Rats (Female-3+3)
Age	**:** 6-8 weeks
Body Weight on Day 0	**:** 150-200gm.
Acclimatization	**:** Seven days prior to dosing.
Veterinary examination	**:** Prior and at the end of the acclimatization period.
Identification of animals	**:** By cage number, animal number and individual marking by using Picric acid.
Number of animals	**:** 3 Female/group,
Route of administration	**:** Oral
Diet	**:** Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water	**:** Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:** The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:** between 22ºC $\pm$ 3ºC.
Relative humidity	**:** between 30% and 70%,
Air changes	**:** 10 to 15 per hour and
Dark and light cycle	**:** 12**:**12 hours.
Duration of the study	**:** 14 Days

Administration of Doses:

***BHRAMASTHIRAM* (BA)** was suspended in water and administered to the groups of wistar albino rats in a single oral dose by gavage using a feeding needle.

The control group received an equal volume of the vehicle.

Animals were fasted 12 hours prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. Three Female animals are used for each group. The dose level of 5, 50, 300 and 2000 mg/kg body weight was administered stepwise. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously as per the guideline after substance administration.

The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. Finally, the number of survivors was noted after 24 hrs and these animals were then monitered for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Limit test

The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. A limit test at one dose level of 2000 mg/kg body weight was carried out with three animals per step. The test substance-related mortality was not produced in animals, so further testing at the next lower level need not be carried out.

Observations:

The Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed.

All observations are systematically recorded with individual records being

maintained for each animal.

a. Mortality

Animals will be observed intensively at 0.5, 2.0, 4.0, 6.0, 12.0, 24.0 and 48.0 hours following drug administration on day 1 of the experiment and daily twice thereafter for 14 days.

b. Body weight

Individual weight of animals was determined before the test substance was administered and weights will be recorded at day 1, 7, and 14 of the study.

Weight changes were calculated and recorded. At the end of the test, surviving animals were weighed and humanly killed.

c. Cage-side observation

These include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, somato motor activity and behavioral patterns. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

d. Gross necropsy

- All animals (including those which die during the test period are removed from the study) will be subjected to gross necropsy.
- Gross necropsy includes examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents, brain, eye, thymus, lungs, heart, spleen, liver, kidneys, adrenals, testes and uterus of all animals.

Histopathology

Microscopic examination will be carried out in organs to show the evidence of any toxicity in gross pathology.

Data and reporting

All data were summarized in tabular form, (Table-23-26) showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test, description of toxic symptoms, weight changes, food and water intake.

Test substance and Vehicle

In order to ensure the uniformity in drug distribution in the medium the suspension was made by mixing *BA* 2% Water solution and it was found suitable for dose accuracy.

Justification for choice of vehicle

The vehicle selected as per the standard guideline was pharmacologically inert and easy to employ for new drug development and evaluation technique [59].

4.3.2. 28-Days Repeated Oral Toxicity (407) Study Of *Bhramasthiram*

28-Days Repeated Oral Toxicity (407) Study of
Bhramasthiram (BA)

Test Substance	: ***BHRAMASTHIRAM (BA*)**
Animal Source	: TANUVAS, Madhavaram, Chennai.
Animals	: Wister Albino Rats (Male -24, and Female-24)
Age	**:** 6-8 weeks
Body Weight	**:** 150-200gm.
Acclimatization	**:** Seven days prior to dose.
Veterinary examination	**:** Prior and at the end of the acclimatization period.
Identification of animals	**:** By cage number, animal number and individual marking by using Picric acid
Diet	**:** Pellet feed supplied by Sai Meera Foods Pvt Ltd,

Bangalore.

Water **:** Aqua guard portable water in polypropylene bottles.

Housing & Environment **:** The animals were housed in Polypropylene cages provided with bedding of husk.

Housing temperature **:** between 22ºC±3ºC.

Relative humidity **:** between 30% and 70%,

Air changes **:** 10 to 15 per hour

Dark and light cycle **:** 12**:**12 hours.

Duration of the study **:** 28 Days.

Groups of animals

GROUPS	No.of Rats
Group I Vehicle control (water)	20 (10 male, 6 female)
Group II Bhramasthiram (2mg /kg)	20 (10 male, 10 female)
Group III Bhramasthiram(10 mg/kg)	20 (10 male, 10 female)
Group IV Bhramasthiram(20mg/kg)	20 (10 male, 10 female)

Justification for Dose Selection:

The results of acute toxicity studies in Wistar albino rats indicated that *BHRAMASTHIRAM* was non-toxic and no behavioural changes was observed up to the dose level of 2000 mg/kg body weight.

On the basis of body surface area ratio between rat and human, the doses selected as per OECD guideline three dose levels were selected for the study.

They are low dose (5X), high dose (10X). X is calculated by multiplying the

acute toxicity dose 2000mg) and the body surface area of the rat (0.018), 5X dose is (10mg/kg), 10X dose is (20mg/kg) The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

Preparation and Administration of Dose:

*BHRAMASTHIRAM***(BA)** at three doses respectively was suspended with water, it was administered to animals at the dose levels of X, 5X, 10X. The test substance suspensions were freshly prepared every two days once for 28 days.

The control animals were administered vehicle only. The drug was administered orally by using oral gavage once daily for 28 consecutive days.

Methodology

Randomization, Numbering and Grouping of Animals:

80 Wistar Albino Rats (40M + 40F) were selected and divided into 4 groups. Each group consist of 20 animals (Male -10 and Female-10). First group treated as a control and other three group were treated with test drug (low, high) for 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

Observations:

Experimental animals were kept under observation throughout the course of study for the following:

Body Weight:

Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study. From the data, group mean body weights and percent body weight gain were calculated.

Food and water Consumption:

Food and water consumed per animal was calculated for control and the treated dose groups.

Clinical signs:

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality:

All animals were observed twice daily for mortality during entire course of study.

Functional Observations:

At the end of the 4th week exposure, 'sensory reactivity' to graded stimuli of different types (auditory, visual and proprioceptive stimuli), 'motor reactivity' and 'grip strength' were assessed.

Laboratory Investigations:

Following laboratory investigations were carried out on day 29 in animals fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Biochemistry and potassium EDTA (1.5 mg/ml) for Hematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

Hematological Investigations

Blood samples of control and experimental rats was analyzed for hemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count and packed cell volume (PCV).

Biochemical Investigations

Serum was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, BUN, creatinine, triglyceride, cholesterol and glucose levels was carried using standard methods. Activities of glutamateoxaloacetatetransaminase/Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

Necropsy:

All the animals were sacrificed by excessive anesthesia on day 29. Necropsy of all animals was carried out.

Histopathology

Histopathological investigation of the vital organs was done. The organ pieces (5-6µm thick) of the highest dose level of 300 mg/kg were preserved and were fixed in 10% formalin for 24 hours and washed in running water for 24 hours. Samples were dehydrated in an auto technique and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Hematoxylin-eosin. The organs included heart, kidneys, liver, ovary, pancreas, brain, spleen and stomach, of the animals were preserved they were subjected to Histopathological examination.

Statistical analysis:

Findings such as body weight changes, water and food consumption, hematology and blood chemistry were subjected to One-way ANOVA followed by dunnet'S multi comparison test using a computer software programme – GRAPH PAD VERSION-3 version. [149]

4.4. PHARMACOLOGICAL ACTIVITY

4.4.1. IN-VITRO ANTICANCER ACTIVITY DETERMINATION BY MTT ASSAY

KB (oral cancer cells), HeLa (cervical cancer cells) was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbeccos modified Eagles medium (DMEM (Sigma Aldrich USA).

The HeLa cell and KB cell line was cultured in 25 cm2 tissue culture flask with DMEM supplemented with 10% FBS L-glutamine, sodium bicarbonate and antibiotic

solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml).

Cultured cell lines were kept at 37ºC in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany).

The viability of cells was evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

Cells seeding in 96 well plate:

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension ($5x10^4$ cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO_2 incubator.

Preparation of plant extracts and compound stock:

1 mg of BA was weighed and dissolved in 1ml of DMEM and dissolved completely by cyclomixer. After that the solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

Anticancer Evaluation:

After 24 hours the growth medium was removed, freshly prepared each plant extracts in 5% DMEM(Dulbecco's modified eagle medium) were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 100µl of 5% MEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO_2 incubator. Not treated control cells were also maintained.

Anticancer Assay by Direct Microscopic observation:

Entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Anticancer Assay by MTT Method:

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization.

After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5% CO_2 incubator for 4 hours.

After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (dimethyl sulphoxide DMSO Sigma Aldrich, USA) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals.

The absorbance values were measured by using microplate reader at a wavelength of 540 nm (Laura B. Talarico et al., 2004) [150].

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD sample x 100}}{\text{Mean OD of control Group}}$$

APPENDIX

Instruments and reagents used:

DMEM media	-	Sigma Aldrich, USA D5648
Fetal Bovine Serum	-	Gibco, US orgin-
0.25% Trypsin	-	Invitrogen, USA 25200-056
Micropipettes	-	F1 Thermoscientific USA
CO_2 Incubator	-	Eppendorf, GERMANY
Phase Contrast Microscope	-	Olympus, JAPAN with Optika Pro 5

		Camera
MTT	-	Sigma Aldrich M5655
ELISA Reader	-	ERBA, GERMANY
Culture Plates and Flasks	-	NUNC, Thermoscientific USA
Image Magnification	-	10X

Figure 20. Microplate Reader

Figure 21. Phase Contrast Microscope

Figure 22. CO_2 Incubator

4.4.2. IN VITRO ANTI- TUMOUR ACTIVITY

Cell culture materials

The human cervical carcinoma a cell line KB cell line, SiHa, used in the study was obtained from National Centre for Cell Science (NCCS), Pune, India. The cells were grown in DMEM containing 2m ML-glutamine supplemented with 10% fetal bovine serum and 100U/ml of penicillin-streptomycin. The cells were incubated in a humidified 5 %CO_2 incubator at37°C.

Cell growth analysis

SiHa cells were seeded at a density of 1 × 105 cells/ml in24-well plates in triplicates. Next day, the cells were dosed with different concentrations of *BA* (0, 10, 20, 40 and 80 g/ml) and grown for 24, 48 and 72 h. The cells were harvested and counted for viability using trypan blue dye exclusion method.

Colony formation assay

The cells were plated at a seeding density of 1 × 103 cells/ ml in 6-well plates. After 24 h, the cells were exposed to various concentrations of *BA*: 0, 10, 20, 40, and 80 g/ ml. Plates were incubated at 37°C in a5%CO_2 incubator for one week.

This was followed by fixing the colonies with 4%paraformaldehyde and staining with0.5%crystal violet [77]. The colonies were photographed with SonyDSC-S75cyber-shotcamera.

Soft agar assay

Control SiHa cells (5×10^3cells/ml) as well as cells treated with different concentrations of *BA* (10-80 g/ml) were mixed at 40°C with 0.35% agarose (DNA grade, GIBCOBRL, CA, USA) in culture medium and gelled at room temperature for 20min over a previously gelled layer of 0.5% agarose in culture medium in 6-wellplates. After incubation for 10days, colonies were photographed directly using an Axiovert200M microscope (CarlZeiss, Germany) and counted.

Measurement of Apoptosis

The cells were plated at a seeding density of 5 × 105 cells/ well and treated with different concentrations of *NMC* (0-80 g/ml). After 24 h of treatment, the cells were harvested and washed with PBS twice. Cells were stained with Annexin V-FITC following the manufacturer's instructions (Annexin V-FITC apoptosis kit #3, In vitro-gen) and analyzed for apoptosis by FACS using Cell Quest Software.

Statistical analysis

All experiments were performed in triplicates and repeated at least five times and the data were presented as mean ±SD. Statistical analysis was conducted with the Sigma Stat3.5program (Systat Software, Inc.) using one- way ANOVA. The level used for comparisons was =0.05.

4.4.3. ANTIOXIDANT ACTIVITY [151,152]

Method:

After the end of sub-acute toxicity study, the intermediate dosage group of animals were sacrificed and organs such as liver and kidneys were excised out and analyzed for oxidative stress markers. The concentration of oxidative stress markers such as Lipid peroxide, Glutathione, Glutathione peroxidase and Catalase were analyzed. Lipid peroxides (Thiobarbituric Acid Reactive Substances – TBARS) in tissues were assayed by the method of Yagi. [84] The colour formation with Thiobarbituric acid (TBA) was used as index. Reduced glutathione (GSH) was estimated by the method of Ellman in which yellow colour developed when Dithionitro-bis-benzoic acid (DTNB) added to the compounds sulfhydryl groups. [85]

Glutothione peroxidase (GPx) estimated by the method of Rotruck et al, 1973 in which H_2O_2 reduced to water whereas organic hydroperoxides reduced to alcohol at the expense of GSH. [86] [87] The activity of Catalase (CAT) was determined by the method of Sinha. [88] In this assay, Dichromate in Acetic acid heated in the presence of Hydrogeperoxide converted to Perchromic acid and then to Chromic acetate. The formed chromic acetate was measured at 620 nm.

The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang et al [2001].

The decrease in the absorption of the DPPH solution after the addition of the sample was measured at 517 nm. Ascorbic acid (10mg/ml DMSO) was used as reference.

PRINCIPLE

1, 1-diphenyl-2-picryl hydrazyl is a stable free radical with pink colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

$$DPPH + [H\text{-}A] \rightarrow DPPH\text{-}H + (A)$$

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

REAGENT PREPARATION

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

PROCEDURE

Different volumes of sample such as 1.25µL - 20µL (12.5 µg/mL- 200µg/mL) from a stock concentration of 10 mg/mL were made up to a final volume of 20µl with DMSO and 1.48ml DPPH (0.1mM) solution was added. A control without the test compound, but an equivalent amount of distilled water was taken. The reaction mixture incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517nm. 3ml of DPPH was taken as control. [151,152]

CALCULATION

$$\% \text{ inhibition} = \frac{control - test}{control} X100$$

5. RESULTS AND DISCUSSION

One of the *Siddha* Herbo-mineral formulation, *Bhramasthiram* has been exposed to several modern scientific studies to establish its efficacy to scientific people and public. The study includes literary collections, physicochemical and phytochemical analysis, instrumental analysis, toxicological study and pharmacological studies are done to justify the Anticancer activity of *Bhramasthiram* against various cell lines study. The drug *Bhramasthiram* has been selected for Anti-cancer activity in reference with the text **"The pharmacopeia of *Siddha* Research Medicines"** Literary collections about the drug from various text books were done. *Siddha* literatures related to the drug bring the evidence and importance of its utility in treating the cancer. [152,153]

The desirable consequences of *Bhramasthiram* are displayed and discussed for its anticancer nature.

From review of literature

Discussion on Gunapadam review

- The general properties& many preparations of Veeram is used to kill certain cancer growth as per *Siddha* classical text.
- Sodium chloride are potential to inhibit the many tumerous growth
- The poem for general properties of Vellai *saranai* directly indicates to cure the cancer and tumour.

Discussion on modern drug review

- Mercuric chloride has cyto-toxic effect [154].
- The bioactive compounds from oxalis cornuculata, which are potential to inhibit cancer cell proliferation and tumour growth.
- *Trianthema decandra* contains anticancer, anti tumour and anti-oxidant activity.
- *Pistia stratiotes* are potential to inhibit cancer cell proliferation.

Discussion on pharmaceutical review

"வேர்பாரு தழைபாரு மிஞ்சினக்கால்
மெல்ல மெல்ல பற்ப செந்தூரம் பாரே"

These lines stressed about potentiality and medicinal values of higher order medicinal (Padhangam) preparation [16].

10 years of shelf life denotes its long time efficacy.

Discussion of pharmacological review

The cell lines for my anticancer activity were HeLa, KB and SiHa. They are the genomes of HPV 16 and HPV 18 respectively. These HPV 16 and HPV 18 are the responsible for 93% of Oral and Cervical cancer [3].

So, the analysis of pharmacological activity through HeLa and SiHa cell lines are the novel methods for validation. They explained about the effective anticancer Activity of *BA.*

Discussion on materials and methods

- The selection of trial drug was taken from the book **The pharmacopeia of *Siddha* Research Medicines**, written by **Dr.M. Shanmugavelu, L.I.M., H.P.I.M.**, was approved by the Department of AYUSH as Per Classical *Siddha* literature.
- This illustrates that ***BA*** is one of the best medicine in *Siddha* system.
- The ingredients were bought from the authenticated vender and they were identified and authenticated by the experts in Post Graduate Department of Gunapadam, GSMC, Chennai. So the ingredients were perfect and original.
- The preparation of medicine was done at the well-equipped lab of the Post Graduate Department of Gunapadam. So the principles of GMP were adhered during the process.
- The analytical parameters were conducted at registered and licensed laboratories only. Thus the result of *Bhramasthiram* under various analytical procedures shows accuracy of it.
- The *Siddha* Herbo-mineral formulation *Bhramasthiram* had been subjected to various studies for its scientific validation and safety assessment. Literary collections, physicochemical and Elemental analysis, Toxicological study, Pharmacological studies are done to prove its efficacy.

Discussion on Standardization techniques

***Siddha* parameters**

The system of regeneration standardizes its medicinal preparations itself by some exclusive ways to ensure the safety and efficacy of them. For the preparatory medicine *Padhangam*, the perfect *Siddha* system handled the following procedure as the part of standardization.

The outcome of *Bhramasthiram* according to *Siddha* standardization techniques are represented here.

Table 13. Results of Siddha standardization

S.No	Paramaeter	Results of ideal Padhangam	Results of BA	Interpretation
1.	Colour	White	White in colour	White in colour.
2.	Floating on water	Floats on water	Floats on water	Lightness of the drug.
3.	Finger print test	Impinged in the furrows of fingers	Impinged in the furrows of fingers	Indicates fine particles of powder.
4.	Lustre	Lustreless	Lustreless	Change of specific metallic character of raw material after Sublimation.
5.	Taste	Nospecific taste	No specific taste	Change of metallic character of raw material after Sublimation.

Colour:

It is White in colour. The absence of shining indicates there is no free form of metals.

Floating on water:

Bhramasthiram floats on water. It is due to its less specific gravity. So, it possesses the property of padhangam.

Figure 24.Floating of water

Finger print test:

Bhramasthiram impinged on the crevices of finger. This indicates the particles are fine and it is in micro size.

Figure 25.Finger print test of *Bhramasthiram*

Lusterless and tasteless:

It is lusterless and tasteless.

Taste:

No specific taste in the *Bhramasthiram.*

- It is due to change of specific mineral character of raw drug material after sublimation.

Table 14. Physical characterization of Bhramasthiram

S.no	Parameter	Result
1.	Colour	White in colour
2.	State of the drug	Powder
3.	Consistency	Fine powder
4.	Solubility	Well soluble in water, DMSO. Sparingly soluble in acids (Hcl and H_2So_4)
5.	Sense on touch	Fine
6.	Sense on taste	Tasteless
7.	Sense of smell	No significant smell is observed

Table 15. Results of Physico chemical analysis

S.no	Parameter	Result
1.	pH	4.07
2.	Specific Gravity	0.973
3.	Loss on drying at 105º Celsius	Less than 1%
4.	Total ash	1.45%
5.	Water soluble ash	Less than 1%
6.	Acid insoluble ash	Less than 1%
7.	Partical size	Completely passes through sieve no.108

Discussion on physic-chemical analysis

Solubility

- Solubility is one of the important parameters to attain desired concentration of drug in systemic circulation the required pharmacological response.

- The oral bioavailability depends on several factors including aqueous solubility, drug permeability etc.
- The most frequent causes of low oral bioavailability are attributed to poor solubility and low permeability [154].
- *BA* is soluble in major solvent, sparingly soluble in some of the solvents thereby it proves its efficiency of solubility in the stomach indirectly, increased in bio-availability.

pH (potential hydrogen):

- *Bhramasthiram* shows acidic ph.
- The P^H is measured of hydrogen ion concentration.It is the measure of the acidic or alkaline nature.7.0 is neutral, above 7.0 is alkaline and below is acidic
- This pH level plays a role in enzyme activity by maintaining the internal environment thus regulating the homeostasis.
- It is also an important factor for drug absorption [155]. Because of the acidic nature, the drug is more readily absorbed in an acid medium like stomach which enhances the bioavailability of the drug.
- The result conclude that the oral bioavailability of the drug *Bhramasthiram* is very high.

Specific gravity

- The trial drug *BA* shows specific gravity which is lesser than water. It shows its nature of absorption.

Loss on drying

- Loss on drying(LOD) gives the total amount of volatile content and moisture(water)present in the drug.
- The stability of a drug and its shelf-life are dependent on moisture content.
- The low moisture content of *BA* indicates that it has long shelf life. Moisture content increased can adversely affect the active ingredient. But, *BA* moisture doesn't damage it. So the low moisture content of *BA* offers maximum microbial stability.

Ash Values

Total Ash value

- Ash is one of the components in the proximate analysis of biological materials consisting mainly of salty, inorganic constituents.
- Low total Ash value of *BA* indicates the richness of organic substances like Sodium, Chloride, Calcium, Potassium. These organic compounds are responsible for the mineral supplements and therapeutic effect of *Bhramasthiram.*

Acid insoluble ash

- Lower the acid insoluble ash value better will be the drug quality [156].
- The drug possesses a low value (Less than 1%) of acid insoluble ash indicating that the preparation did not contain any sand, dust and stones.

Water soluble ash

- Decreased water soluble ash value (Less than 1%) indicates easy facilitation of diffusion and osmosis mechanisms.
- This nature might be helpful for the better absorption

RESULT OF BIO CHEMICAL ANALYSIS

Table 17. Results and acidic radical studies:

S.No	Parameters	Result
1	Test for Chloride	Positive
2	Test for Nitrate	Positive

Table 17. Result of Basic radicals

Sl.no	Parameter	Result
1	Test for Potassium	Positive
2	Test for Calcium	Positive
5	Test for Sodium	Positive
6	Test for Iron (Ferrous)	Positive
7	Test for Zinc	Positive
10	Test for Copper	Positive
11	Test for Mercury	Positive

The biochemical analysis reveals the presence of chloride, nitrate, potassium, calcium, sodium, iron, zinc, copper, mercury.

DISCUSSION:

Numerous in vitro and vivo studies have suggested favourable effects of several vitamins and minerals play vital role on angiogenesis, immunity, cell differentiation, proliferation, and apoptosis.

Nitrates

Nitrates and nitrites occur naturally in fruit and vegetables, which are regarded as an important part of a healthy diet due to the powerful evidence of beneficial health effects against cancer. Nitrates play an important role inhibition of tumour growth. [157]

Calcium:

More recent studies reveal that calcium and vitamin D3 reduce the aggregation of the cancer. Vitamin D, and has been found to participate in regulating apoptosis, cell proliferation, and differentiation. Many in vitro and vivo studies have suggested favourable effects of calcium on protective effects against many cancer types, including colorectal, breast, endometrial, prostate and ovarian. However, in this study, there was relationship between reduce the risk of HNC and calcium supplement. [158]

Sodium:

Sodium has cytotoxic effect. Increased sodium level depresses the cancer cell growth. [159]

Iron:

- Iron plays an important role in forming complexes with molecular oxygen in hemoglobin and myoglobin. These two compounds are general oxygen transport proteins in vertebrates.
- Iron is also important in the process of cellular respiration oxidation and reduction in plants and animals.
- Iron-containing enzymes, proteins and heme prosthetic groups participate in many biological oxidations and in transport. [160]

Zinc:

Zinc is needed for immune function, wound healing and blood clotting. Some experiments show that the Zinc slows the growth of cancer cells in the laboratory.

Mercury:

Miles (1926) introduced perchloride of mercury as an antiseptic agent in rectal surgery. Goligher et al. (1951), Morgan (1955) and Keynes (1961) introduced the technique of flushing the colon and rectum in restorative cancer surgery. Royle (1964) described alleged mercury intoxication after using 200 ml of 1: 500 perchloride of mercury solution as an anti-cancer agent in renal surgery.

In all cases the uptake of mercury into the blood has been well below the toxic levels defined by Lane (1954). Studies of the urinary output have confirmed the safety of this technique. It is therefore concluded that mercury perchloride is a safe anti-cancer agent when used as described in large bowel surgery. H Brendan Devlin et al.

Chloride:

Chloride has cytotoxic effects. the presence of these radicals helps *BA* for its therapeutic effect. The human CLCA2(calcium activated chloride channels regulator 2) enhances chloride in breast cancer cells and reduces pH to 6.7. This

observation gives some chloride channels are able to promote apoptosis by reducing intracellular p^H.

CFTR is involved in multiple molecular pathways that modulate cell inflammation and apoptotic signaling, so it is possible that mutations in this gene could also modify the risk of development of cancer. Mutations in the *CFTR* gene could also have a protective role in some tumours such as lung cancer, melanoma, colon, and breast cancer. Furthermore, low expression of *CFTR* polymorphisms may contribute to a reduced risk of prostate cancer. [161]

Copper

Copper plays important Role in inhibition of proteasome activity, reduction of androgen receptor (AR) protein expression, suppression of cell proliferation, and induction of apoptotic cell death were observed in both prostate cancer cell lines treated with CQ-Cu complex, but not with CQ alone. Furthermore, animal studies of mice bearing human C4-2B xenografts showed that treatment with CQ (10 mg/kg/day) for 30 days resulted in significant inhibition of tumor growth (66%) compared to the control, associated with proteasome inhibition, induction of apoptosis, suppression of AR expression, and inhibition of angiogenesis in CQ-treated tumor tissues.[162]

Copper with iron

The transition metals such as iron (II) and copper (I) oxidized by H_2O_2 can generate the hydroxyl radical (Fenton Reaction). It is suggested that inhibition of the antioxidant enzymes and induction of apoptosis and necrosis in cancer cells caused by the ROS production can be a potential treatment for cancer. [163]

Potassium

Overexpression of the G-protein–activated inwardly rectifying potassium channel GIRK1 (KCNJ3) is correlated with the presence and degree of breast cancer lymph node metastases (Stringer et al., 2001). The pharmacological inhibition or genetic suppression of potassium channels reduces growth in multiple cancer types (Pardo and Stühmer, 2014; Urrego et al., 2014. Induction of the expression and activity of a constitutively open mutant sodium channel also leads to rapid and robust cell killing

in multiple tumor cell types (Lemaire and Halperin, 2009. . In hyperkalemic diseases (Parkinson, Addison) have reduced cancer rates. [158]

Zinc

The regulatory effects of zinc on the NF-kB pathway makes zinc highly significant in the prevention of cancer cell growth patterns. Zinc is associated with inhibiting angiogenesis in tumor cells as well as the secretion of inflammatory cytokines.

Zinc deficiency can promote a variety of human cancers including esophageal, cervical as well as cancers related to the digestive tract, head, and neck. Zinc supplementation has been shown to reduce the number of tumours and carcinogenic severity.

Zinc is especially important in prostate, Bladder, skin and breast cancers. Zinc is observed to lower inflammation, suppress abnormal tissue growth, and lower the incidence of larger skin lesions and more deleterious tumours Zinc has been found to stimulate apoptosis in abnormal cells.[164]

AVAILABILITY OF MICROBIAL LOAD

DISCUSSION:

These herbo-mineral drug are prepared from plant material they are prone to contamination. The contamination of herbo-mineral drugs by micro-organism not only cause bio deterioration but also reduces the efficacy of drugs.

The toxic effects produced by microbes makes the herbo mineral drugs unfit for human consumption because the contaminated drug may develop unwanted disease instead of disease being cured.

Here, the contaminations of Padhangam have been examined by bacterial and fungal load.

- Total bacterial load in 10^{-4} dilution is 8 and in 10^{-6} dilution is 1.
- Total fungal load in 10^{-2} dilution is nil and in 10^{-3} dilution is nil.

Here, the contamination of *BA* is within the WHO norms. Hence, the drug is collected, prepared, stored and packed and decontaminated prior to formulation.

Table 18. Result of Anti-Microbial load

Bacteria		**Fungai**	
10^{-4}	**10^{-6}**	**10^{-2}**	**10^{-3}**
8	1	Nil	Nil

INSTRUMENTAL ANALYSIS:

FTIR (FOURIER TRANSFORM INFRA RED SPECTROSCOPY)

Fig No: 25.Peak values of FTIR

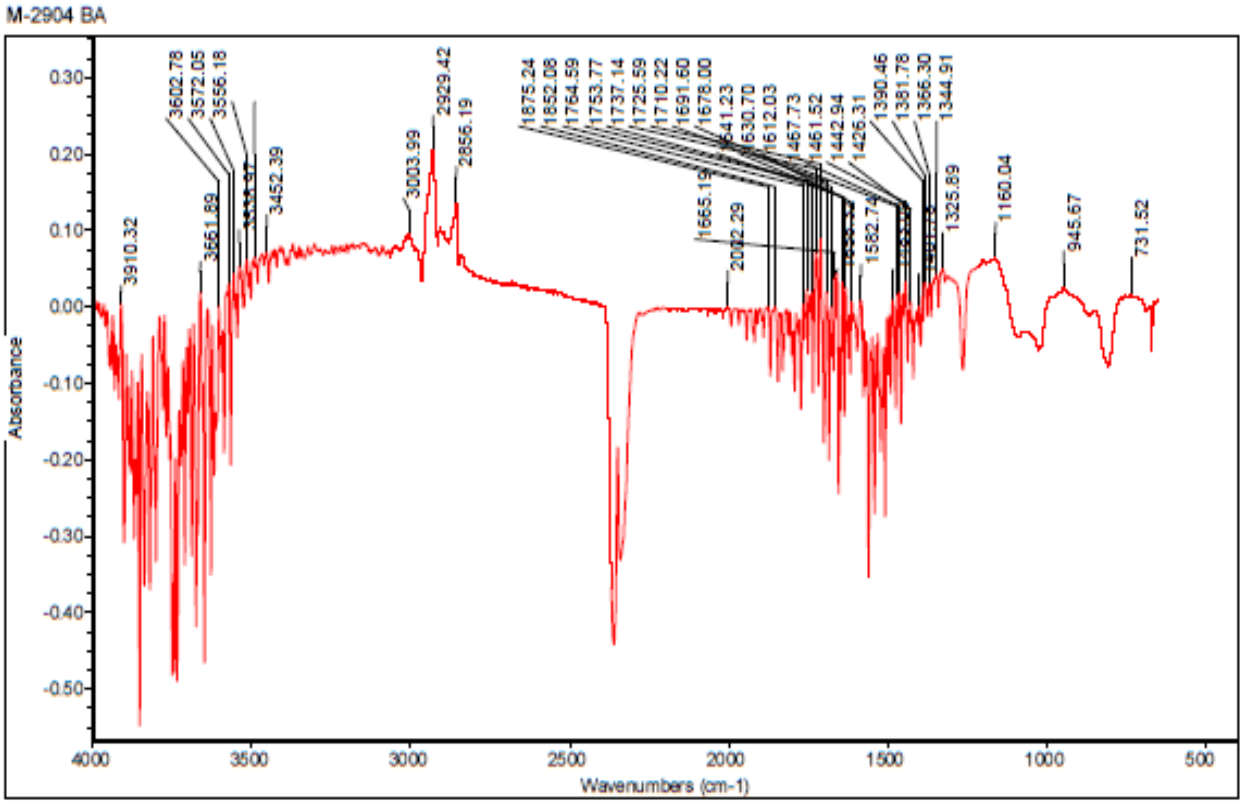


Table 20. Result of FTIR analysis of Bhramasthiram

S.no	Absorption peak(cm^{-1})	Stretch	Functional group
1	3452	N-H	Alkenes,1º,2ºamines, aromatics
2	3003	C-H	Aromatics
3	3556	O-H	Alchol,Phenol
4	2929	C-H	Alkenes
5	1764	C-C	Carboxylic acid
6	1753	C-O	Esters,Saturated,Aliphatic
7	1737	C-O	Esters,Saturated,Aliphatic
8	1725	C-O	α,β Unsaturated aldehyde Aliphatics
9	1710	C-O	α,β Unsaturated aldehyde ketones
10	1691	C-O	α,β Unsaturated aldehyde ketones
11	1678	C-C	Alkenes,
12	1641	C-C	Alkenes, 1º,2ºamines
13	1630	C-C	Aromatics
14	1467	C-H	Alkanes
15	1461	C-C	Aromatics
16	1442	C-H	Alkanes
17	1426	C-C	Aromatics
18	1665	C-C	Alkenes
19	2002	C-H	Alkenes
20	1658	C-Br	Alkyl halide
21	1582	C-C	Aromatics
22	1483	C-C	Aromatics
23	1401	C-C	Aromatics
24	1325	C-N	Aromatic amines
25	1390	C-H	Alkanes
26	1381	C-H	Alkanes
27	1366	C-H	Alkanes
28	1344	N-O	Nitro carbons
29	1160	C-N	Aliphatic amines
30	945	O-H	Carboxylic acid
31	731	O—C	Alkyl halides

DISCUSSION:

- The wavenumbers from 4000 cm-1 to 1500 cm-1 gives details for identification of functional group.
- The wavenumber from 1500 cm-1 to 400 cm-1 provides particulars about molecular fingerprint.
- The above result showed the presence of functional group like alcohols, phenols, alkanes, alkenes,1°amines, aliphatic amines, Alcohol, carboxylic acid, ester, ethers, alkyl halides in *BHRAMASTHIRAM*.
- They may be responsible for the presence of anticancer action of *BA* in Oral and cervical cancer.

Phenols

- Phenols of *BA* possess highly Anti-oxidant property which enhances its effect against the disease.
- The effect of phenols is currently of great awareness due to their anti-oxidative and possible anti-carcinogenic activities.
- Phenolic compounds play an important role in prevention and treatment for cancer and other human disease. Phenolic compounds from medicinal herbs contain phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones, and others.
- Various bioactivities of phenolic compounds are responsible for their chemo preventive properties (e.g., antioxidant, anti-carcinogenic, or anti-mutagenic and anti-inflammatory effects, anti-ulcer, anti-spasmodic and anti-depressant) and also contribute to their inducing apoptosis by arresting cell cycle, regulating carcinogen metabolism and ontogenesis expression, inhibiting DNA binding and cell adhesion, migration, proliferation or differentiation, and blocking signaling pathways.[165]
- A major focus has been the inhibitory effects of phenolic on the stress-activated NF-KB and AP-1 signal cascades in cancer cells which are regarded as major therapeutic targets.
- Phenolic compounds can enhance the body's immune system to recognize and destroy cancer cells as well as inhibiting the development of new blood vessels

(angiogenesis) They also attenuate adhesiveness and invasiveness of cancer cells thereby reducing their metastatic potential.

- Free radicals react easily with phenols to abstract the hydrogen atom from the OH group.
- Phenolic acid and flavonoids also work as reducing agents, free radical scavengers and quenchers of single oxygen formation (Ali Ghasemzadehet al.2011)
- Phenols are the most important groups of secondary metabolites and bioactive compounds.
- Hydroquinone is one of the phenolic group inhibits the free radical reactions. (cho7Alcohol HTI) They are also an antioxidant substance capable of scavenging free superoxide radicals, anti-aging and reducing the risk of cancer.

Alcohol

OH group of *BA* has higher potential towards inhibitory activity against microorganisms.

Alkanes

Alkane derivate like bis (4-amino-5-mercapto-1,2,4-triazol-3-yl) possess anti-cancer activity. [166]

Carboxylic acid

- Benzene-poly-carboxylic Acid Complex (BP-CI) is a novel anticancer complex against human cancer cells.
- Docosahexaenoic acid (DHA) is an omega-3 fatty acid. Its structure is a carboxylic acid (-oic acid) with a 22- carbon chain (Docosa-is Greek for 22) and six (hexa-) cis double bounds[167].
- DHA was revealed to increase the efficacy of chemotherapy in prostate cancer cells and a chemo protective effect in a mouse model was reported.
- It may also be used as a non- toxic adjuvant to increase the efficacy of chemotherapy.
- In mice, DHA was found to reduce growth of human colon carcinoma cells
- The cytotoxic effect of DHA was caused by decrease in cell growth regulators.

Alkyl halide:

High proportion of low molecular weight alkyl halides may be weakly carcinogenic .

Aldehydes

Aldehyde dehydrogenes (ALDH) have great value as prognostic indicator for many tumour and cancers including lung, breast ovarian, pancreatic, and colon and prostate cancer. ALDH+ cells were capable of regenerating prostate cancer tumor cell types in culture and in mouse xenograft. ALDH1A1 caused decreased phosphorylation/activation of CHK1 (Ser 317), PARP and Fanconi Anemia DNA repair pathway proteins and increased γH2AX levels in response to carboplatin treatment in ovarian cancer cells lines.

Loss of ALDH1A1 also disrupted the cell cycle profile of ovarian cancer cells. These findings indicate that ALDH1A1 enhances activation of DNA strand break resistance and repair, suggesting an important role for this protein in resistance to DNA damage induced by X-ray irradiation and certain chemotherapeutic drugs..[168]

Ether:

- Certain ether lipids such as 1-0-octadecyl-2-0 methyl-rec-glycero-3-phosphocholine represent a new class of anti -neoplastic agents. These ether lipids have been shown to be cytotoxic for a wide variety of tumours.

Aromatics:

- Aromatics oils contain the chemical constituent limonene and related terpenoids, which have been shown to be effective in the treatment of cancer. [169]

Essential oil

Garlic contain major organo sulphur compounds(OSCs) such as diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl trisulfide (DATS), these were used as experimental materials to investigate their modulation effects on cell viability and cell cycle in human liver tumor cells (J5). According to the results of DATS significantly decreased the cell viability as compared with the control Garlic and onion contains aromatics and essential oil. These oil were responsible for cancer.

A recent study found odds ratios among persons with high *versus* low intakes of garlic and onions that correlated with starkly reduced risk of colorectal adenoma [51]. Persons who consume a high proportion of garlic decreasing susceptibility to stomach and colon cancers [52]. human studies reveal garlic's anti-tumorigenic potential in stomach, colonrectal, breast, lung, and endometrial cancers. Very limited evidence supports a relation between garlic consumption and reduced risk of colon, prostate, esophageal, larynx, oral, ovary, or renal cell cancers. [170]

XRD DISCUSSION:

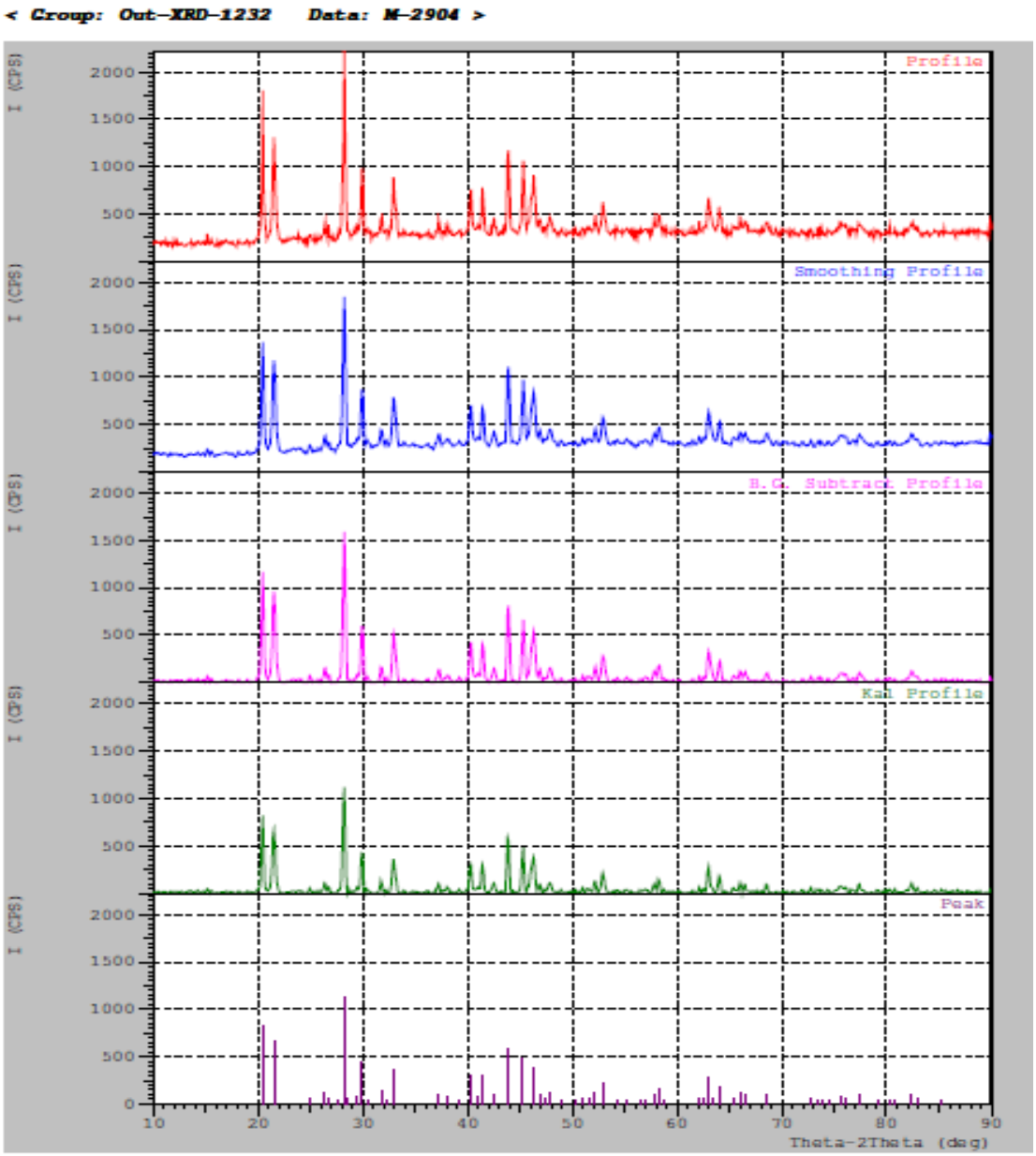


Figure 26: XRD Image of *Bhramasthiram*

The crystalline structure, the size and shape of the particles are highly dependent on the route of synthesis and highlights the efficacy of the drug. The nanomaterial characteristics may enhance bio absorption of the drug. XRD pattern of *Bhramasthiram* shows the good crystallinity after sublimation process. The major diffraction peaks are identified after XRD analysis BA concluded that HgS in Nano crystalline range (21-28) is association with organic molecules probably plays an important role in making it biocompatible and nontoxic at therapeutic doses.

Other elements present in *BA* act as additional supplement and possible helps in increase the efficacy of the formulation and provide evidence supporting an electrophilic hypothesis of carcinogenesis [171].

SEM (Scanning Electron Microscope):

The following image is done by magnification via aperture shows maximum depth focused. Zoom magnification denotes rapid surveying of the specimen.

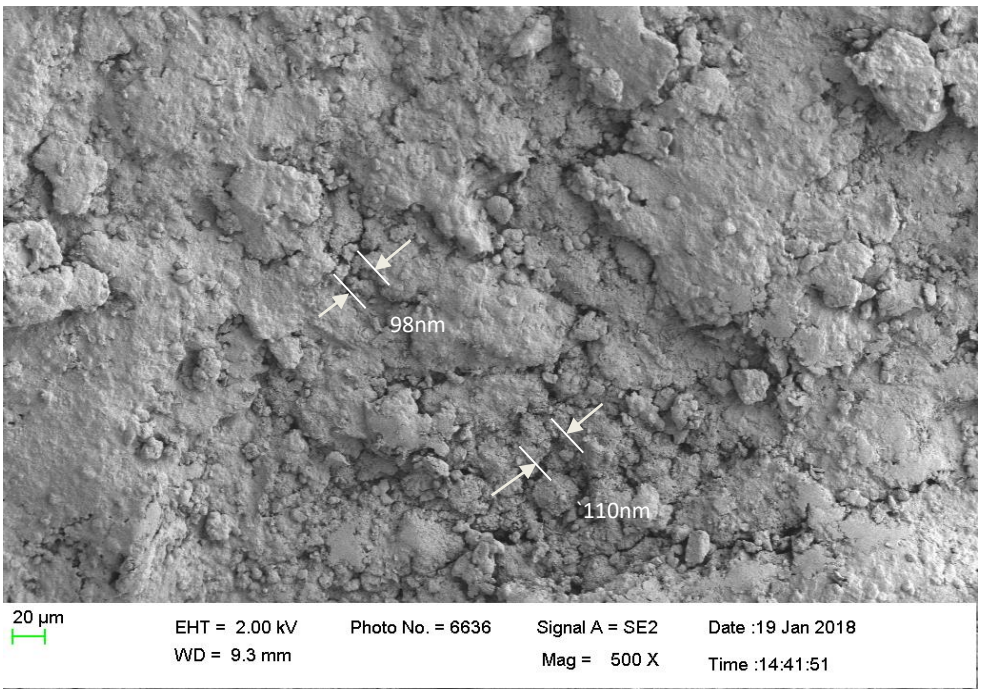


Figure 27: XRD Image of *Bhramasthiram*

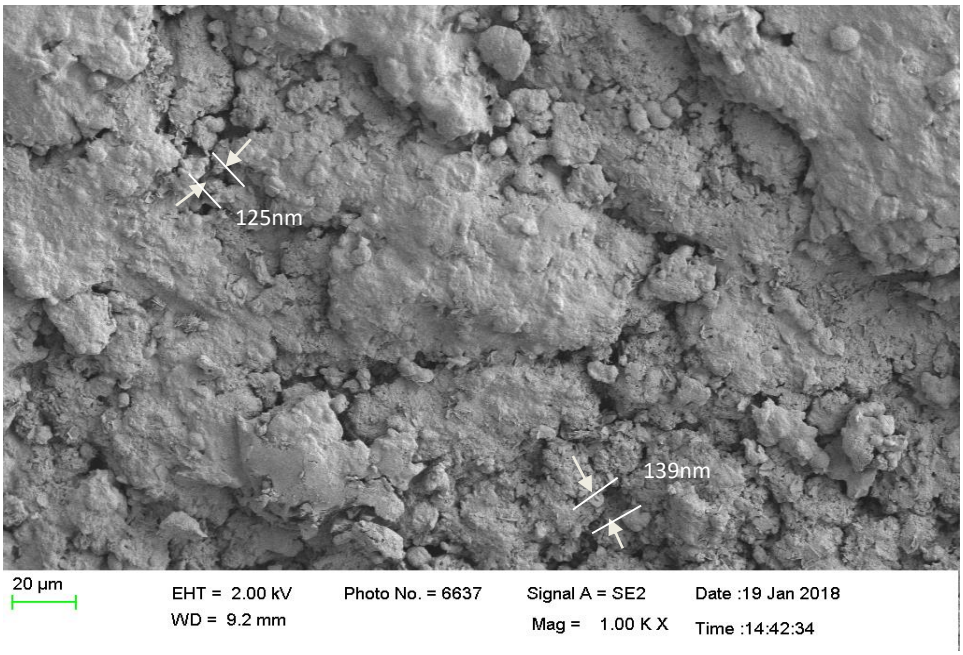


Figure 28: XRD Image of *Bhramasthiram*

Discussion on SEM reports

In addition, the particle size and chemical elements were assessed by Scanning Electron Microscope SEM is one of the most widely used instruments in research areas The above SEM study shows of microscopic resolution of 5.00kx and 10.00kx showed objects of sizes ranging from 20µm.The difference in morphology as evident from the micrograph is due to presence of various substances in the sample. Size and surface of micro particles can be easily manipulated to achieve both passive and active drug targeting. They control the release of drug during the transportation and at the site of localization, alters drug distribution in the body and subsequent clearance of the drug so as to achieve increased drug therapeutic efficacy thereby bio-availability and reduced side effects.

Advantages of Nanoparticles:

- Enhancing solubility of hydrophobic drugs.
- Prolonged circulation time,
- Preventing undesirable side effects,
- Minimizing nonspecific uptake,
- Specific cancer targeting [172]
- Improving intra cellular penetration

- The test drug *Bhramasthiram* contains Nano particles. Thus it enhances bioavailability and facilitates absorption.

Nano technology is a promising way from cancer management towards cancer elimination.

Nano particles have high surface area to that of volume ratio thereby it facilitates the attachment of functional groups and binding to tumour cell.

The particles of Nano size show that the drug can easily enter into the cells at the molecular levels to treat the disease rapidly and increase the therapeutic effect.

ICP-MS RESULTS OF DISCUSSION

Table 20.Results of ICP-MS

S.no	Elements	Detectable levels
1.	Arsenic	BDL
2.	Mercury	0.993mg/L
3.	Cadmium	BDL
4.	Lead	BDL
5.	Selenium	1.3mg/L

Table 21. AYUSH guidelines limit for heavy metals is as follows

Heavy metal limit	Maximum permissible
Arsenic (As)	3 ppm (3 mg/kg)
Cadmium (Cd)	0.3 ppm (0.3 mg/kg)
Lead (Pb)	10 ppm (10 mg/kg)
Mercury (Hg)	1 ppm (1 mg/kg)

***ICP-MS interpretation of BA*:**

In the results of heavy metal analysis, the trial drug BA shows Arsenic, Cadmium, Lead in below detectable level. But there was a mild presence of mercury in a level of 0.993mg/L, because of the content of trial drug.

Mercury:

Miles (1926) introduced per chloride of mercury as an antiseptic agent in rectal surgery. Gallagher (1951), Morgan (1955) and Keynes (1961) introduced the technique of flushing the colon and rectum in restorative cancer surgery.

Royle (1964) described alleged mercury intoxication after using 200 ml of 1: 500 per chloride of mercury solution as an anti-cancer agent in renal surgery. In all cases the uptake of mercury into the blood has been well below the toxic levels defined by Lane (1954).

Studies of the urinary output have confirmed the safety of this technique. It is therefore concluded that mercury per chloride is a safe anti-cancer.So it is in large bowel surgery [173].

Selenium

Selenium is a powerful mineral.it plays a crucial role in cell defene against cancer. Selenium also protect the body against contaminates such as mercury, cadmium and silver, speed the elimination of cancer cells and slow tumour growth.

It also plays a role in recycling antioxidants, such as vitamin e, then lower risk of cancer by preventing free radicals from damaging cells.

Selenium supplement decreased risk and growth rate of tumour. [174] Selenium supplement may also be able to halt the growth of polyps in the colon and reduce the risk of lung and liver cancer. [175][176]

TOXICOLOGICAL STUDIES

Acute oral toxicity in rats

Observation done:

Table no: Dose finding experiment and its behavioral signs of Toxicity for *Bhramasthiram*

Table 22. Acute toxicity behavioral signs of Bhramasthiram

SL	CONTROL GROUP	OBSERVATION	TEST GROUP	OBSERVATION
1	Body weight	Normal	Body weight	Normal
2	Assessments of posture	Normal	Assessments of posture	Normal
3	Signs of Convulsion, Limb paralysis	Normal	Signs of Convulsion, Limb paralysis	Absence
4	Body tone	Normal	Body tone	Normal
5	Lacrimation	Normal	Lacrimation	Normal
6	Salivation	Normal	Salivation	Normal
7	Change in skin color	No significant color change	Change in skin colour	No significant color change
8	Piloerection	Normal	Piloerection	Normal
9	Defecation	Normal	Defecation	Normal
10	Sensitivity response	Normal	Sensitivity response	Normal
11	Locomotion	Normal	Locomotion	Normal
12	Muscle gripness	Normal	Muscle gripness	Normal
13	Rearing	Mild	Rearing	Mild
14	Urination	Normal	Urination	Normal

Table 23. Gross behavior of animals

No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	Control	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
2.	2000mg	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-

1.*Alertness 2. Aggressiveness 3. Pilo erection 4. Grooming 5. Gripping 6. Touch Response 7. Decreased Motor Activity 8. Tremors 9 Convulsions 10. Muscle Spasm 11. Catatonia 12. Musclerelaxant 13. Hypnosis 14. Analgesia15.Lacrimation 16. Exophthalmos 17. Diarrhoea 18. Writhing 19. Respiration 20. Mortality*

Table 24.*Body Weight Observation of Acute toxicity in Wister albino rats exposed *Bhramasthiram

DOSE	DAYS		
	1	7	14
CONTROL	280.2±42.30	281.4 ± 64.12	282.6 ±26.18
HIGH DOSE	280.3± 21.24	280 ± 4.64	280.4 ± 2.86
P value (p)*	NS	NS	NS

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table 25.*Water intake(ml/day) of Wistar albino rats group exposed to *Bhramasthiram

DOSE	DAYS		
	1	7	14
CONTROL	61 ± 1.12	62±2.22	63.9±1.14
High DOSE	62.4±4.4	63±1.14	63.8±2.12
P VALUE(P)*	NS	NS	NS

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table 26.*Food intake (gm/day) of Wistar albino rats group exposed to *Bhramasthiram

DOSE	**DAYS**		
	1	7	14
CONTROL	56.24±2.22	56.2±7.42	58.4±3.46
High DOSE	55.6±1.63	55.7±2.62	55.1±5.38
P VALUE(P)*	NS	NS	NS

*N.S- Not Significant, **(p >0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test*

Acute toxicity discussion:

- In the acute toxicity study, the rats were treated with different concentration of *BHRAMASTHIRAM* from the range of 2mg/kg to 2000mg/kg.
- However, behavioural changes, body weight, water intake, food intake does not produce much significant thus the result is not significant.
- These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract.
- In acute toxicity test drug *BHRAMASTHIRAM* was found to be non-toxic at the dose level of 2000mg/ kg body weight.

REPEATED DOSE 28-DAYS ORAL TOXICITY STUDY OF *BHRAMASTHIRAM*

Table27.* Body weight of wistar albino rats group exposed to *Bhramasthiram

DOSE	DAYS		
	1	15	28
CONTROL	280.2±10.03	280.2 ± 10.24	280.6 ± 24.61
LOW DOSE(2 mg)	281.6 ± 11.20	281.5 ± 16.14	280.8± 52.10
MIDDOSE(10 mg)	280.8 ±12.10	280.9 ±18.9	281.7 ± 8.60
HIGH DOSE(20 mg)	280.5± 7.10	280.8 ± 8.30	280.9 ± 04.32
P value (p)*	NS	NS	NS

*NS- Not Significant, ** (p> 0.01)<,*(p >0.05), n = 20 values are mean ± S.D (One way ANOVA followed by Dunnett's test*

Table28: **Water intake (ml/day) of Wistar albino rats group exposed to *Bhramasthiram***

DOSE	DAYS		
	1	15	28
CONTROL	98.4 ± 1.25	98.3 ± 1.02	98.8±2.30
LOW DOSE(2 mg/kg)	98.2 ± 6.40	98.3 ± 1.50	98.5±1.14
MID DOSE(10 mg/kg)	98.8 ± 3.40	98.9 ± 9.06	98.9 ± 6.5
HIGH DOSE(20 mg/kg)	99.6 ± 1.30	99.5 ± 2.20	99.9±6.52
P value (p)*	NS	NS	NS

*NS- Not Significant, **(p > 0.01),*(p >0.05), n =20 values are mean ± S.D (One way ANOVA followed by Dunnett's test*

Table 29. Food intake (gm/day) of Wistar albino rats group exposed to *Bhramasthiram*

DOSE	DAYS		
	1	15	28
CONTROL	190.4 ± 2.25	191.03±6.14	191.3±4.20
Low dose(2mg/kg)	191.2±1.12	191.2±1.02	191.4±4.14
Mid dose(10mg/kg)	190.9 ± 3.65	190.7 ± 2.05	190.5 ± 8.23
High dose(20mg/kg)	189.6 ± 1.20	189.2±4.21	189.5±6.40
P value (p)*	NS	NS	NS

*NS- Not Significant, **(p > 0.01),*(p >0.05), n = 20 values are mean ± S.D (One way ANOVA followed by Dunnett's test*

Table 30. **Haematological parameters of Wistar albino rats group exposed to *Bharamasthiram***

Category	Control	Low dose (2mg/kg)	Mid dose (10mg/kg)	High dose (20mg/kg)	P value (p)*
Haemoglobin(g/dl)	10.2±0.24	10.10±0.36	9.11±0.26	8.28±0.26	*
Total WBC ($\times 10^3$ l)	14.52±0.05	14.62±0.13	14.68±0.07	15.40±6.16	N.S
Neutrophils(%)	26.15±0.01	27.11±0.22	27.12±0.26	28.20±2.30	N.S
lymphocyte (%)	79.10±1.06	81.23±1.02	82.13±1.03	82.26±4.46	N.S
Monocyte (%)	0.9±0.03	0.8±0.05	0.8±0.06	0.9±0.07	N.S
Eosinophil(%)	3.2±0.04	3.6±0.06	3.7±0.08	3.8±0.08	N.S
Platelets cells10^3/µl	604.16±2.66	601.10±4.26	594.09±3.24	590.06±4.6	*
Total RBC 10^6/ µl	8.49±0.01	8.47±0.50	7.07±0.25	6.04±0.32	N.S
PCV%	37.65±0.6	38.30±1.32	38.72±0.5	38.56±2.24	N.S
MCHC g/dL	38.4±1.42	38.26±0.47	38.16±0.43	38.34±2.24	N.S
MCV fL(µm^3)	54.04±4.60	53.06±3.43	53.05±2.43	53.34±2.14	N.S

*N.S- Not Significant, ** (p < 0.01), *(p <0.05), n = 20 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table 31. Liver Function Test of Wistar albino rats group exposed to *Bhramasthiram*

Treatment	T Bilirubin (mg/dL).	Triglycerides (mg/dL)	Cholesterol (mg/dL)	Protein	SGPT/ALT (U/L)	ALP (U/L)
Control	0.50±0.07	66.24±7.74	66.61±4.45	7.14±0.12	89.64±6.57	215.08±10.48
Low dose **(2mg/kg)**	0.57±0.16	68.34±6.22	64.10±6.46	6.84±0.24	91.11±3.39	230.46±1.68
Mid dose **(10mg/kg)**	0.58±0.44	68.22±6.33	63.03±0.44	6.57±0.42	93.65±5.46	231.36±3.99
High dose **20mg/kg)**	0.65±0.15	70.24±9.32	59.12±2.43	6.36±0.42	94.31±1.23	233.40±6.96

*N.S- Not Significant, ** (p < 0.01), *(p <0.05), n = 20 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table32. Bio chemical Test of Wistar albino rats group exposed to *Bhramaasthiram*

Biochemical Parameters	**Control**	**Low dose (2mg/kg)**	**Mid dose (10mg/kg)**	**High dose (20mg/kg)**	**P value (p)***
Glucose (r) (mg/dl)	123.64±10.24	123.08±9.24	123.32±9.14	123.4±2.47	N.S
T.cholesterol(mg/dl)	66.61±4.45	65.10±6.26	66.05±5.23	67.12±2.43	NS
Trigly(mg/dl)	66.24±7.74	68.34±6.22	68.34±6.22	68.34±6.22	N.S
LDL	72.4±2.14	72.13±2.54	70.66±3.12	70.42±10.2	NS
VLDL	11.24±1.30	11.20±2.21	11.10±1.21	11.16±12.16	NS
HDL	27.14±6.12	28.42±2.30	28.46±3.20	29.17±2.14	NS
Ratio 1(T.CHO/HDL)	3.41±1.16	3.32±1.40	3.36±1.36	3.54±2.03	NS
Ratio 2(LDL/HDL)	1.92±1.14	1.95±1.12	1.93±1.16	1.96±09.02	NS
Albumin (g/dL)	5.43±0.16	5.49±0.52	5.48±0.46	5.46±9.48	NS

*NS- Not Significant** ($p < 0.01$), * ($p<0.05$), n = 20 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table 33. Renal function test of Wistar albino rats group exposed to Bhramasthiram

Parameters	**Control**	**Low dose (2mg/kg)**	**Mid dose (10mg/kg)**	**High dose (20mg/kg)**	**P value (p)***
Urea (mg/dl)	26.70±0.19	27.60±0.26	27.36±0.35	28.18±1.24	N.S
Creatinine(mg/dl)	0.22±0.02	0.38±0.04	0.57±0.06	0.63±0.07	N.S
Bun(mg/dl)	21.10±0.20	23.16±0.90	25.18±0.90	27.74±1.22	NS
Uric acid(mg/dl)	6.04±0.34	7.03±0.51	7.35±0.90	8.42±0.20	N.S

*NS- Not Significant, ** ($p < 0.01$), * ($p <0.05$) , n = 20 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Sub-acute toxicity discussion:

Results

Observations

Overall observations were similar in both sex rats.these studies were done with certain doses like low dose(2mg),5x(10mg),10x(20mg).The values are in non significant.

Clinical signs of toxicity

No clinical signs of toxicity were observed.

Mortality

No mortality was observed after 28 days repeated dose administration of *BA*. All animals survived to study termination period.

Body weight

Increased in body weights were compared to their initial weight. No significant alterations were observed in body weight.

Food and water consumption

No effect of treatment was noted with in the the non-significant in result.

Physiological activities

No changes in the general behaviours

Blood analysis

a. **Hematology**

   No treatment related effects were observed. However, there is a slight variation in the result.

b. **Biological parameters**

c. There is a slight difference has been noted but it is normal within the limit. No treatment related effects were observed

d. Histological examination

- Histopathology studies were carried out on liver, kidney and spleen and recorded. Blood samples for hematological and blood chemical analyses were taken from common carotid artery.
- All rats were sacrificed after the blood collection. The internal organs and some tissues were observed for gross lesions. All tissues were preserved in 10% neutral buffered formaldehyde solution for histopathological examination
- Histological examination of organs did not show as much pathological changes.

Sub-acute toxicity discussion:

- The repeated 28days oral toxicity studies of *BA* showed did not produced any toxicity signs in wistar albino rats. Daily administration of BA at different doses 2mg/kg,10mg/kg,20mg/kg for 28 days was tolerated by the rats without any mortality and morbidity, indicates the drug tolerance. Dose selected for the Sub acute toxicity study was 10mg, 20mg/kg of *BHRAMASTHIRAM.*

- No physical changes were observed throughout the doing period. No mortality was observed during the whole experiment.

- No abnormal deviations were observed. No significant changes were observed in the values of different parameters studied when compared with controls and values obtained were within normal biological and laboratory limits.

- No significant changes in red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), Erythrocyte sedimentation rate (ESR) in all the treated groups as compared to respective control groups.

- Hence the herbo mineral formulation of *BA* can be considered to be safe drug for prolonged duration use as reveled by toxicological studies.

Discussion

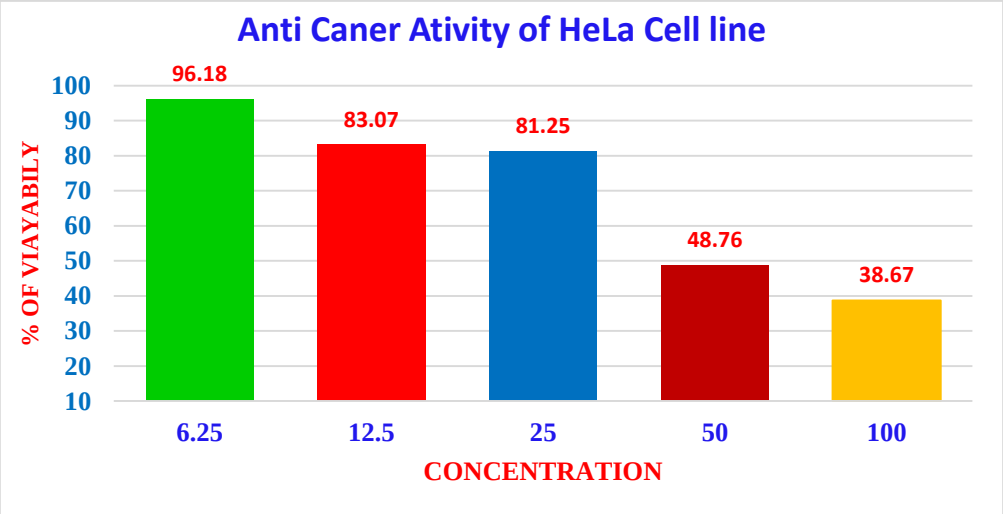
The above studies show histopathology slides of sub-acute toxicity. Even though there is less toxic effect seen in the vital organs. after administration of the test drug *Bhramasthiram* thus the safety of the drug is revealed, so that it can be administered for long time without any side effect.

PHARMACOLOGICAL ACTIVITIES

Table 34. ANTI-CANCER ACTIVITY OF CELL LINE- HeLa CELL LINE

Sample Concentration (µg/mL)	**OD value I**	**OD value II**	**OD value III**	**Average OD**	**Percentage Viability**
Control	0.5898	0.6278	0.6013	0.6063	100.00
Sample code: BA					
6.25	0.5812	0.5836	0.5847	0.5832	96.18
12.5	0.5033	0.5064	0.5013	0.5037	83.07
25	0.4919	0.4926	0.4933	0.4926	81.25
50	0.2929	0.2984	0.2956	0.2956	48.76
100	0.2829	0.2874	0.2756	0.1535	38.67

IC 50 ***BA shows the 50% incubatory concentration at 50µg/ml.*** Graph-1

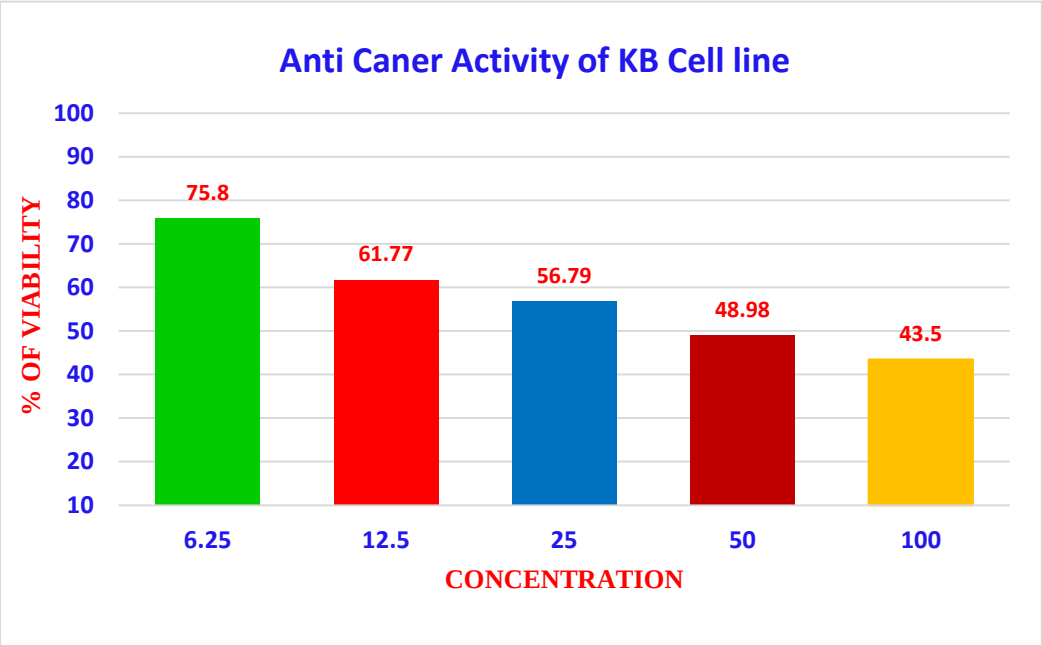


Graph-1

Table 35. ANTI-CANCER ACTIVITY OF CELL LINE- KB CELL LINE

Sample Concentration (µg/mL)	**OD value I**	**OD value II**	**OD value III**	**Average OD**	**Percentage Viability**
Control	0.3859	0.3922	0.3642	0.3808	100
Sample code: BA					
6.25	0.2852	0.2919	0.2888	0.2886	75.80
12.5	0.2259	0.2437	0.2361	0.2352	61.77
25	0.2143	0.2126	0.2219	0.2163	56.79
50	0.1736	0.2040	0.1820	0.1865	**48.98**
100	0.1626	0.1685	0.1659	0.1657	43.50

IC 50 ***BA shows the 50% incubatory concentration at 50µg/ml.***



Graph-2

Graph -1 and 2 shows the drug dose and % of Inhibition of HeLa, KB cells after the Bhramasthiram extract treatment. It can be observed by the result of MTT assay that the IC dose of Bhramasthiram is 50µg/ml. As the dose increases the HeLa cell & KB cell viability decreases. And also It was found that the % of growth inhibition increasing with increasing concentration of Bhramasthiram steadily up to 6.25 µg/ml on HeLa cell line (Table1&2-and graph -1, graph2) and that IC value on HeLa cell line was 50 and R value was 0.6063 and IC value on a KB cell line was 50 and R value was 0.3808

Cytotoxic effect by MTT assay:

- MTT is a yellow water soluble tetrazolium salt. Succinate dehydrogenase, a mitochondrial enzyme in living cells, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. therefore, the amount of formazan produced is directly proportional to the number of viable cells.

- This assay is based on the metabolic reduction of 3- (4, 5- dimethylthiazol-2-yl) -2, 5-difeniltetrazol (MTT) by mitochondrial enzyme succinate dehydrogenase in a coloured compound blue (formazan), allowing to determine the functionality of the mitochondrial treated cells.

- This method has been widely used to measure survival and cell proliferation. The amount of living cells is proportional to the amount of formazan produced.

- Cell lines derived from NCCS, Pune were free from any kind of bacterial and fungal contamination.

- To determine the cytotoxic effect of novel *Siddha* formulation ***Bhramasthiram*** against the Hela and KB cell lines. The experiment was screened at different concentrations to determine IC50 using MTT assay a chart was plotted using the % cell viability in Y axis and concentration of the test sample in X-axis.

Bhramasthiram at different doses (6.5-100µg in100 µl of 5% MEM) was administered for 24 hrs. It was found that the number of cells decreases as the dose increases and at approximately 50 µg/ml dose of extract, 50% of the cells (Hela,KB) were less as compared to normal control as shown in figure (). The percentage of cells viability was determined by calculating the O.D of treated against the control. Reading optical density (OD) is performed in a spectrophotometer at a wave length of 540nm. Comparison values are made on a basis of 50% inhibition of growth (IC_{50}) in treated cells with specific agents. Results are tabulated in Table34&35- and schematically represented in Graph-1 &2

Analysis of Membrane Morphological Characteristics by Haematoxylin /Eosin (H/E) Staining

Morphological changes such as changes to the cell membrane, loss of membrane asymmetry and cell shrinkage, are the early stage of apoptosis was analyzed by H/E staining. The IC dose (50µg/ml) treated cancer cells show features of apoptosis whereas treated with same amount of dose, to normal treated cells appeared without any significant changes.

- Since the discovery of the Cisplatin anti-tumor activity, great efforts have focused on the rational design of metal-based anticancer agents that can be potentially used in cancer chemotherapy.
- Over the last four decades, a large number of metal complexes have been extensively investigated and evaluated *in vitro* and *in vivo* .
- The key focuses of these studies lie in finding novel metal complexes which could potentially overcome the hurdles of current clinical drugs including toxicity, resistance and other pharmacological deficiencies.
- Herbo-mineral and Minerals compounds have been used in medicine for several thousands of years.
- The medicinal uses and applications of metals and mineral complexes are of increasing clinical and commercial importance. Monographs and major reviews, as well as dedicated volumes, testify to the growing importance of the discipline [177].

- The field of inorganic chemistry in medicine may usefully be divided into two main categories: firstly, ligands as drugs which target metal ions in some form, whether free or protein-bound; and secondly, metal-based drugs and imaging agents where the central metal ion is usually the key feature of the mechanism of action.
- Several pieces of evidence indicate that iron deprivation could be an excellent therapeutic approach:
  (i) dietary iron restriction markedly decreases tumour growth in rodents.
  (ii) The antibodies which block transferrin-binding to cellular receptors inhibit cancer cell growth *in vitro* and *in vivo* [178][179]
- Oncologists and scientists engaged in the research of cancer treatments should conduct a comprehensive study on the efficacy of mercuric perchloride which is being used as an anti-cancer drug in the age old *Siddha* system [180].
- Three years of research has shown that metal (mercury, arsenic and copper) based *Siddha* drug is a safe alternative for Cisplatin therapy or arsenic trioxide in selected cases of cancer treatments wherein the patients cannot bear the adverse effects. He found that mice treated with *Siddha* drugs showed better health than what did in cisplatin therapy in terms of appetite, haemoglobin, red blood cells and white blood cells [181].

- Miles (1926) introduced perchloride of mercury as an antiseptic agent in rectal surgery. Goligher (1951), Morgan (1955) and Keynes (1961) introduced the technique of flushing the colon and rectum in restorative cancer surgery.
- Royle (1964) described alleged mercury intoxication after using 200 ml of 1: 500 perchloride of mercury solution as an anti-cancer agent in renal surgery. In all cases the uptake of mercury into the blood has been well below the toxic levels defined by Lane (1954).
- Studies of the urinary output have confirmed the safety of this technique. It is therefore concluded that mercury perchloride is a safe anti-cancer agent used as described in large bowel surgery.
- Studies have shown that phenols present in herbal plants such as *Trianthema decandra, pistia stratiotes, oxalis cornuculata* have cytotoxic effects on

different tumours. Mechanisms of these compounds are carried out through apoptosis.

- Thus from the above study, it is evident that the cytotoxic property of *Bhramasthiam* may be due to the synergistic interactions between the mineral complex and plant derivatives.

ANTI-TUMOUR ACTIVITY

Discussion

To test the effect of on the growth kinetics, SiHa cells were treated with different concentrations of BA:0,10,20,40 and 80µg/ml and were grown for 24,48 and 72h. At the end of each treatment, the cells were stained with trypan blue, and the viable cells that excluded the dye were counted. It was observed that there was a dose-dependent decrease in the growth kinetics of BA treated cells compared to the untreated control cells (Fig.10A).

Moreover, it was found that at around 80 µg/ml concentration of BA treatment, there was a significant decrease (~2-fold) in the growth kinetics compared to that observed in the untreated control cells ($p \leq 0.05$ for 24h; $p \leq 0.001$ for 48h and 72h).

This was further confirmed by colony forming assay wherein at a lower seeding density, cells were treated with different concentrations of BA for one week. At 80 µg/ml concentration of BA, the cells exhibited relatively lesser colonies compared to the control cells (Fig.10B).

Consistent with the slow growth rate, it was observed that BA extract induced a dose-dependent decrease in the number of soft agar colonies. Interestingly, at 80 µg/ml BA treatment, the number of soft agar colonies was reduced by ~3-fold ($p \leq 0.001$) compared to the untreated control cells (Fig10C.).

All these data indicated that BA altered the growth kinetics of SiHa cells in a significant manner that could be a positive indicator for testing its anti-tumor activity in cervical cancer cells.

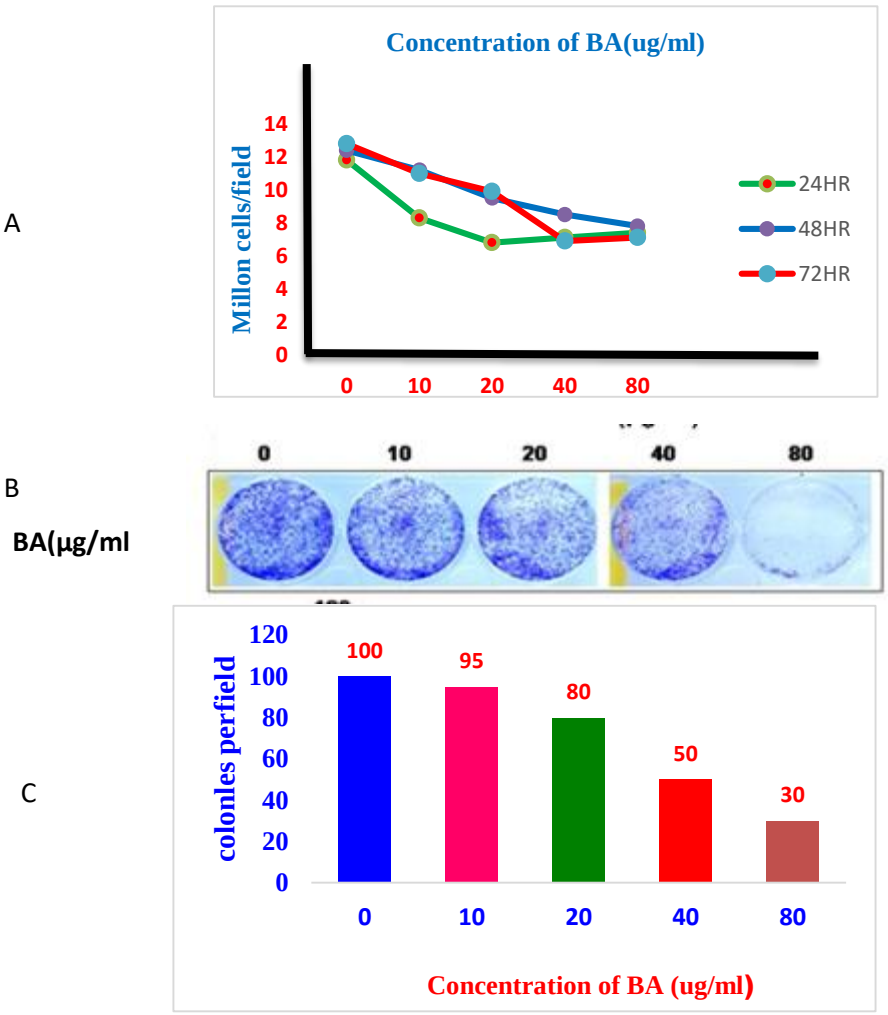


Figure 28-ANTI-TUMOUR ACTIVITY OF *BA*

Figure 28 *BA* alters growth kinetics of cervical cancer cells. (A) The cells were treated with various concentrations (0-80 µg/ml) of *BA* for 24, 48 and 72 h, and the number of viable cells were counted using the trypan blue dye exclusion assay. The growth kinetics has been presented in the figure taken at different time points. Data represent mean ± SD of five different experiments. . (B) The cells (1 × 103/ml) were grown in 6-well plates and treated with various concentrations (0-80 µg/ml) of *BA* for one week. The cells were then stained with crystal violet and photographed. The experiments were repeated five times. (C) The cells (5×10^3) were treated with various concentrations (0-80 µg/ml) *BA* and grown in soft agar for 10 days, and the colonies were counted. Colonies were counted from at least 10 different areas, and the average of each is plotted. The data rep- resent mean ± SD of five independent experiments.

Apoptosis

To further elucidate the anti-cancer mechanism of *BA* in cervical cancer cells, we performed apoptosis studies. After treating the cells with different doses of *BA*, the percent apoptotic cells were assessed by Annexin V-FITC and propidium iodide staining, followed by flow cytometric analysis(Figure.26). It was observed that at concentrations of 40 and 80µg/ml *BA*, there was a significant increase in the percentage of cells undergoing apoptosis. Interestingly at 80µg/ml *BA* concentration, there was ~2.6-fold($p \leq 0.001$) increase in the population of cells undergoing apoptosis compared to the untreated control cells.

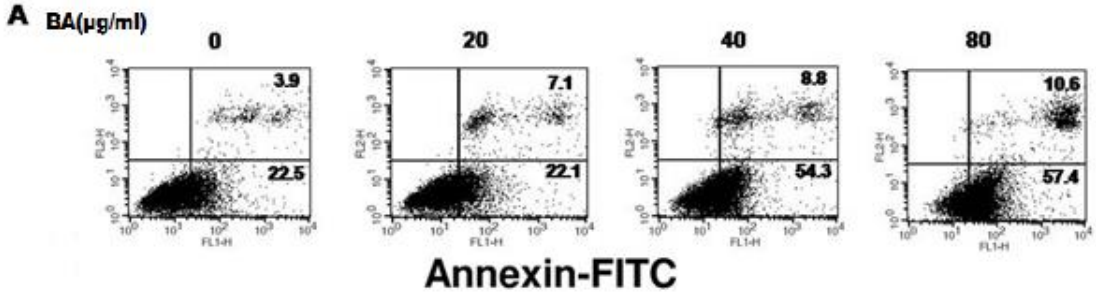
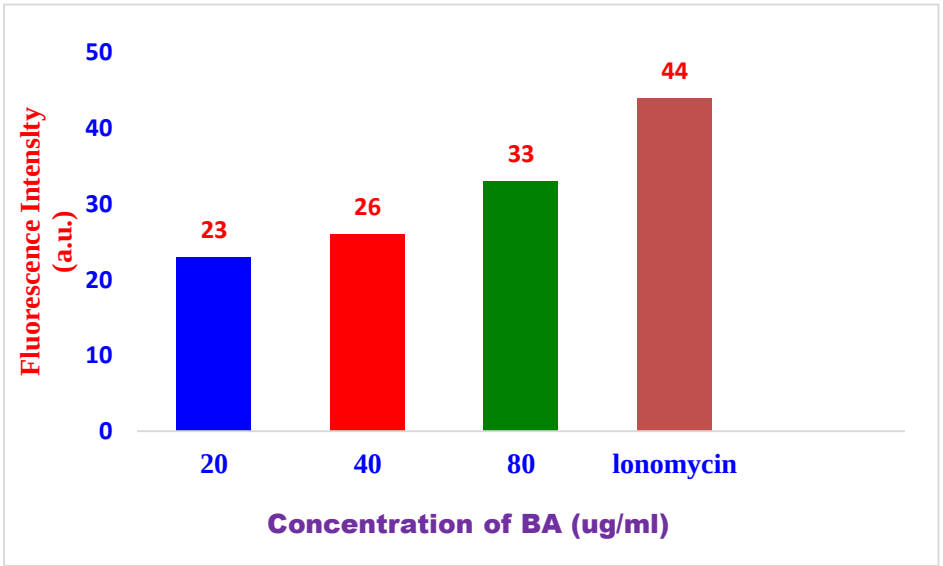


Figure.29 Apoptosis



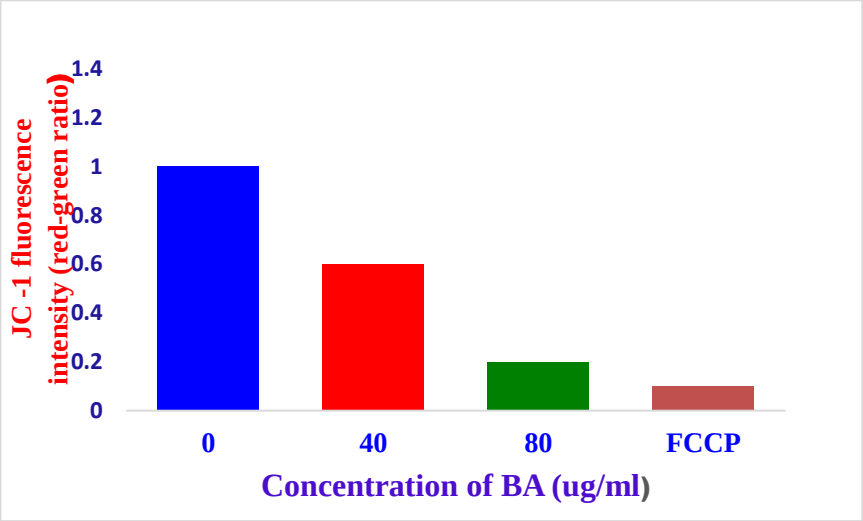


Figure 29 *BA* induces apoptosis in SiHa cells through dysregulation of mitochondrial membrane potential. (A) SiHa cells were treated with different concentrations of BA (0-80 µg/ml) followed by Annexin V-FITC and PI staining to analyze the effect of BA in apoptosis. This was determined by FACS analysis showing the percentage of early (lower right quadrant) and late (upper right quadrant) apoptotic cells. (B) Flow cytometric analysis of the rapid calcium release in SiHa cells.

Conclusion

Bhramasthiram showed promising anticancer activity in oral cancer cell lines killing cancer cells by apoptosis. Further, *Bhramasthiram* demonstrated promising anti-tumor activity in the growth kinetics of HeLa and SiHa cell in without significant toxicity to normal tissues underscoring the pre-clinical efficacy of *Bhramasthiram*, as a potential anti-cancer therapeutic candidate for cancer management.

ANTIOXIDANT ACTIVITY: (IN VITRO STUDY)

- Antioxidants are substances that can prevent or slow damage to cells caused by free radicals, unstable molecules that the body produces as a reaction to environmental and other pressures. They are sometimes called "free-radical scavengers."
- Antioxidants are said to help neutralize free radicals in our bodies, and this is thought to boost overall health.

- Antioxidants can protect against the cell damage that free radicals cause, known as oxidative stress.

Free radicals and their chemical reactions:

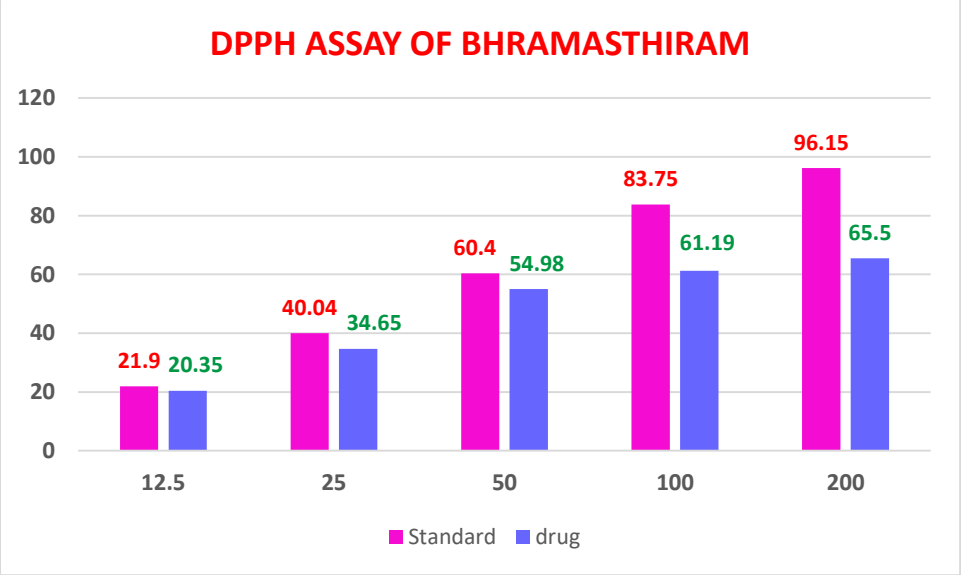
- A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals.
- Radicals are weakly attracted to a magnetic field and are said to be paramagnetic. Many radicals are highly reactive and can either donate an electron to or extract an electron from other molecules, therefore behaving as oxidants. As a result of this high reactivity, most radicals have a very short half-life (10^{-6} seconds or less) in biological systems, although some species may survive for much longer.
- Oxidative stress has been linked to heart disease, cancer, arthritis, stroke, respiratory diseases, immune deficiency, emphysema, Parkinson's disease, and other inflammatory or ischemic conditions.

Result:

Table 36. DPPH assay on BHRAMASTHIRAM

Concentrations (µg/ml)	**Absorbance**		**Percentage of inhibition**	
Bhramasthiram	**Drug**	**Standard**	**Drug**	**Standard**
Control	0.3896	1.7983	-	-
12.5	0.3103	1.4044	20.35	21.90
25	0.2546	1.0782	34.65	40.04
50	0.1754	0.7121	54.98*	60.40**
100	0.1512	0.2921	61.19	83.75
200	0.1344	0.0692	65.50	96.15

**µg/ml: microgram per millilitre. Drug: BA (12.5-200µg/µl). Standard: Ascorbic acid (10mg/ml DMSO)*



IC50 Value – GC- 165.728µg/ml (Calculated ED50 PLUS V1.0 Software)

Discussion on Antioxidant activity in DPPH assay:

- DPPH assay were used for the determination of antioxidant activity of the different extracts. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of BA extract.
- The antioxidant molecules can quench DPPH free radicals by providing hydrogen atom or by electron donation and a colorless stable molecule 1, 1 diphenyl-2- picrylhydrazil is formed and as a result of which the absorbance at 517nm of the solution is decreased.

- In the present study, the *Bhramasthiram* extract was analyzed was able to decolorize DPPH and the free radical scavenging activity was expressed as the percentage decrease in absorbance and Ascorbic acid (10 mg/ ml DMSO) was used as a reference and result was expressed as the percentage decrease in absorbance. In the present study, the extract of *BA* was found to possess concentration dependent scavenging activity on DPPH radicals.

- The values of DPPH free radical scavenging activity of the *BA* extract were given in (Table.33) expressed in the percentage. The extract of *BA* showed the highest DPPH scavenging activity (65.50%) at 200µg/ml and the lowest

percentage of inhibition (20.35%) at 1.25µg/ml. Ascorbic acid (Standard) showed highest percentage of inhibition (96.15%) at 200µg/ml and the lowest percentage of inhibition (21.90%) at 1.25µg/ml.

- This indicated that % of inhibition increased with increase in concentration of both the standard and *BA* extract. The *BA* extract has less free radicle scavenging activity compared to the standard. From the present study, it was concluded that the *BA* extract has good anti-oxidant activity at higher concentrations.

- It is known that oxidative stress induced cell damage not only through damage to proteins, lipids and DNA. It may also alter signaling pathways redox sensitive to changes involved in the response of apoptosis.

- The antioxidants are currently the subject of many studies because, in addition to some interest in the preservation of comestibles, they could be useful in the prophylaxis and treatment of diseases in which oxidative stress is implicated. Many studies realized on natural products have proven that they are especially phenolic compounds who are responsible for their antioxidant activity.

- Several studies have shown the link between the traditional drug formulations rich in antioxidants and the incidence of certain diseases such as **cancer**, diabetes, heart disease and other diseases related to aging.

- Phenolic compounds could prevent cancer by the action antioxidant and / or the modulation of several functions of proteins. Phenolic compounds can prevent carcinogenesis by affecting the molecular events in the triggering, promotion and progression stages.

- Some phenolic compounds (phenolic acids, flavonoids, quinones, coumarins) have proved an effective antioxidant activity and also had anticancer activities/ anti-carcinogenic/ anti-mutagenic.

- Here, the reactive oxygen species (ROS) may be the triggers apoptotic process. In recent years they have been described numerous properties of these

compounds such as the ability to inhibit cell cycle, proliferation cellular and oxidative stress, and induce detoxification enzymes, apoptosis, and stimulate the immune system.

From the present study, it was concluded that the *BA* extract has a marked low level antioxidant activity at higher concentrations. Antioxidant capacities of the extracts were expressed in terms of IC50 value of the extracts and low IC50 value corresponds to a high antioxidant capacity.

Hence the in vitro study of DPPH assay free radicle scavenging activity shows a less antioxidant property and IC50 value (**90.5584µg/ml g/ml**) of the extracts are high in level. So it need different assays and in vivo studies to evaluate the antioxidant property of *BA.* It is therefore hypothesized that *Bhramasthiram* of its antioxidant power could “to repair" Cancer cells.

6. CONCLUSION

The most ancient wisdom and science of life, Siddha, has a long history and its basic principles are valid even today. Less or minimal effectiveness and severe toxic side effects of current cancer therapies draw the global attention towards herbo-mineral medicine to arrest the insidious nature of this disease. In addition, more than 80% of the world's population cannot afford modern medicines. Western medicine provides symptomatic treatment and largely ignores the underlying conditions, whereas Siddha medicine treats the disease from root of origin. Siddha plays an important complementary role to western medicine in treatment efficiency.

Cancer is the second most common malignancy found among men and women worldwide and some are highly resistant to radiotherapy. The other chemotherapy drugs also deliver intolerable side effects which are worse than the disease. This paved way for a novel anticancer drug which cures cervical, oral cancer in a non-invasive way.

The intention of this study is to provide a solution for the above need. For a nonviolent anticancer drug to cervical, oral cancer, ***Bhramasthiram*** was chosen from the Siddha literature as a trial drug "**The pharmacopeia of Siddha Research Medicines**" written by **Dr.M. Shanmugavelu, & Dr.G.D. Naidu L.I.M.,H.P.I.M.** to validate the safety and its efficacy of drug to treating Anti-cancer,anti-tumour,and anti-oxidant activity in animal model.

The trail drug was subjected various studies through which the efficacy of the drug is proved.

The procedure for drug preparation and its techniques for standardization revealed GMP. The trial drug *BA* has satisfied all parameters of testing protocol for Padhangam which was assigned by AYUSH. It showed the accurate production and potency of *Bhramasthiram.*

Physico-chemical analysis the drug shows revealed better bio-availability and richness of its mineral content. Favouring this study the biochemical analysis shows

the presence of inorganic matters sodium, potassium, calcium, zinc, iron, chloride are influences the anti-cancer activity.

FTIR analysis revealed the presence of O-H Groups C-C,N-H group, which indicates the functional groups present in the sample.FTIR interprets the moleculate structure of the sample.

SEM analysis showed the size of the drug in micro particles which denotes that the trial drug could have potent drug delivery.

XRD analysis disclosed the percentage of elements presence in the drug.

Heavy metal analysis shows Mercury present with in the permissible amount, and other elements such as Lead, Cadmium, Arsenic are absent which indicates the purity of the drug and also the safety of the drug.

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The anti-microbial activity of trial drug was also considered for its potential.

Under OECD guidelines, the acute and 28 days repeated oral toxicity studies proved the safety of *Bhramasthiram* at particular dose level. It is very useful in therapeutic dose determination.

The pharmacological studies concluded that the drug are effective has anticancer effect on HeLa cell lines and KB cell lines anti-tumour effect on SiHa cell lines and quantitative measurement of antioxidants by DPPH assay

Factors like safety, efficacy, long self like, bio-availability, presence of significant elements, anions and cations and minerals favouring the activity justifies the main perspective of this study.

Bhramasthiram anti-cancer effect could be validated scientifically. Due to its Non-toxic anti-cancer effect, it would benefit the health community and the world.

7. SUMMARY

Trial drug ***Bhramasthiram*** was selected from the classic literature "**The pharmacopeia of Siddha Research Medicines**" written by **Dr.M.Shanmugavelu, L.I.M.,H.P.I.M.** for its anti-cancer, anti-tumour, anti-oxidant activities.

The dissertation started with an introduction explaining about the Siddha concept, prevalence of cervical, oral cancer and role of the test drug in treating cervical, oral cancer.

The ingredients of the test drug was identified and authenticated by siddha experts. The drug was prepared as per the procedure and subjected to various studies to reveal its potency and effectiveness against the disease.

The physico chemical shows potency of the Bhramasthiram.

The biochemical analysis reveals the presence of chloride, Nitrate, Potassium, Calcium, Sodium, Iron, Zinc, Copper, Mercur.Thus from these results we come to know the effectiveness of the drug is due to the presence of these constituents and it has a synergistic effect in acting against the disease.

The FTIR analysis construe the result that showed the presence of functional groups like alcohol, alkanes, esters, aromatics amines which might be responsible for the presence of anti-cancer action of the drug. SEM picture described its morphology and the particle size.

- Toxicological study was made according to OECD guidelines comprising both acute and repeated oral dose 28days toxicity studies in wistar albino rats. It showed the safety of the drug which attributes its utility in long time administration.

- Pharmacological studies were completed. It revealed the anti-cancer, anti-tumor and anti-oxidant activities of *Bhramasthiram.*
- Results and discussion gives the essential validations to prove the potency of the drug.
- Conclusion gives a Compiled form of the study and explains the synergistic effect of all the key ingredients and activities that supports the study.

8. FUTURE SCOPE

The trial drug ***Sadhakuppai Chooranam*** has its own potency in treating Anxiety, Depression and Catalepsy in animal model which has been established in this study. An incredible action of this drug value against the disease of Neurosis has been revealed from this study of *Sadhakuppai Chooranam.* This must be implicated in the future clinical studies and preclinical chronic toxicity studies. So it could be used worldwide in treatment of Neurosis.

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INGREDIEANTS OF BHRAMASTHIRAM

A.Hydragyrum perchloride

B.Pistia Stratiotes

C.Oxalis cornuculata

D.Sodium chloride

E. Trianthema decandra

Fig.No. Ingredients of *Bhramasthiram*

PURIFICATION OF HYDRAGYRUM PERCHLORIDE

A.Aagayathamarai

B.Veeram

C.Sealding

D.Inginition

E.After purified Veeram

PROCESS OF UPPU PARPAM

F. Salt

G.Trianthema decandra

H.Sealding

I. Iginition (1st process)

J.Incineration

K. Uppu parpam(1th process end)

L.Vellai saranai

M.Sealding

N.Incineration

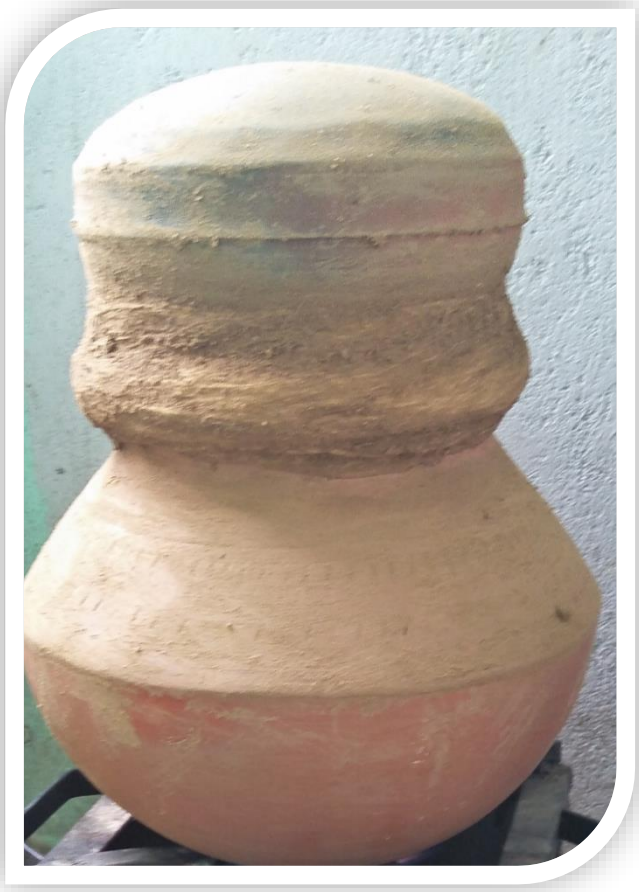
O. Uppu parpam(10th process end)

P. Uppu parpam with puliyarai

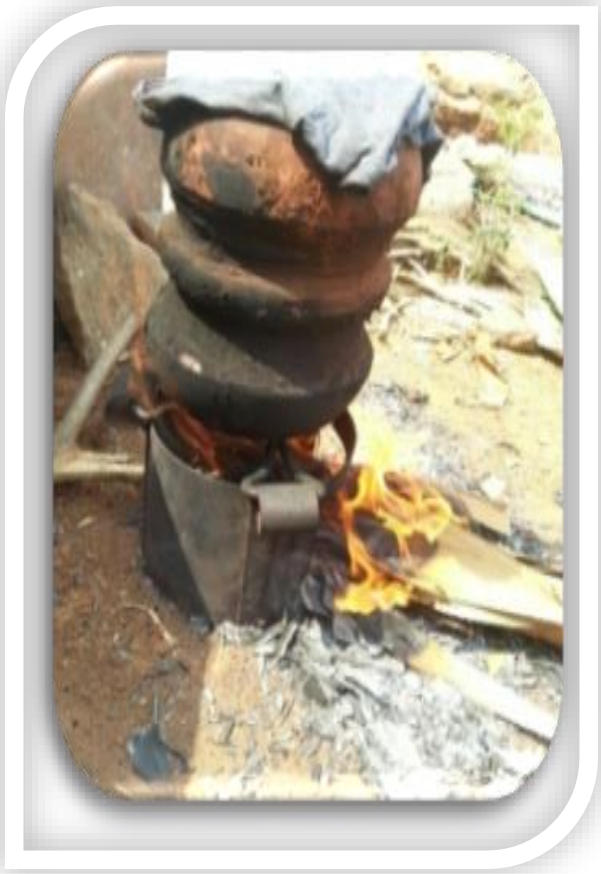
Q.Grinding

R.Veeram is covered by Uppu

S.Pasting upper pot



T. Sealding



U.Incineration

W.Sublimation

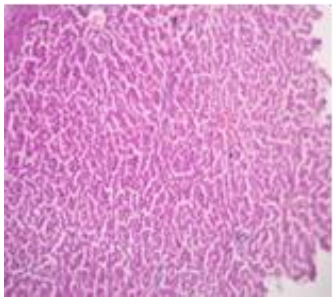
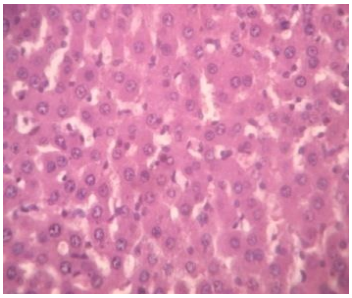
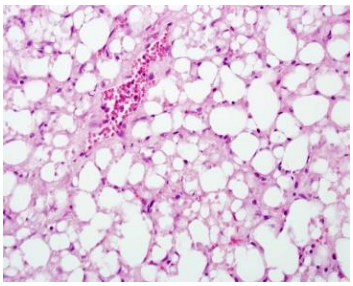
X.End product

Figure (a-x) preparation of *Bhramasthiram*

HISTOPATHOLOGY SLIDES

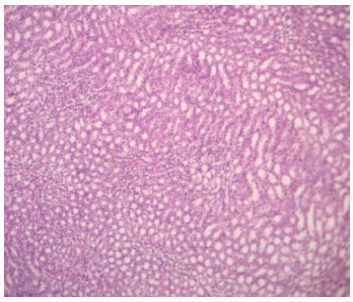
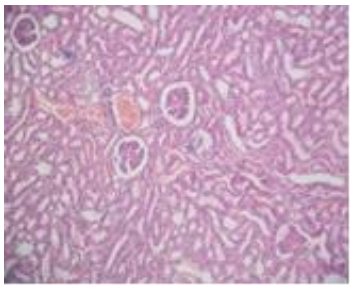
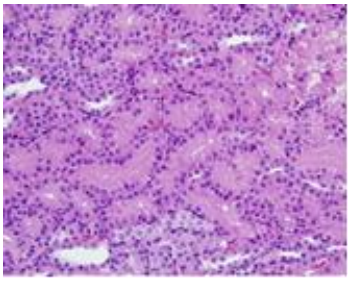
LIVER

Control *BA 2mg* *BA 20mg*

KIDNEY

Control *BA 2mg* *BA 20mg*

SPLEEN

Control *BA 2mg* *BA 20mg*

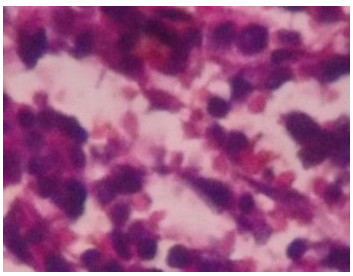
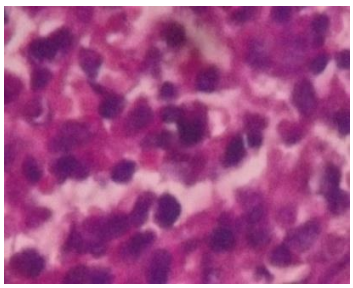
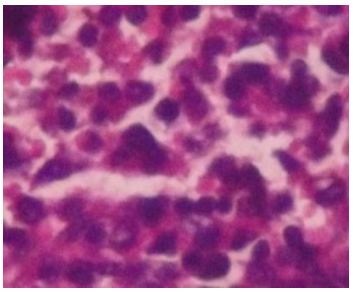




Figure.23 Histopathological slides

Government Siddha Medical College

Arumbakkam, Chennai _ 600 106

CERTIFICATE

Certified that the samples submitted for identification by Dr.S.Semalatha PG Scholar, Department of *Gunapadam*, Government Siddha Medical College, Arumbakkam, Chennai-600 106, were identified as:

Ingredients:

1.*Hydragyrum perchloride* (*Veeram*)

2.*Sodium chloride* (*Kariyuppu*)

3.*Trianthema decandra* (*Vellai saranai*)

4.*Oxalis cornuculata* (*Puliyarai)*

5.*Pistia stratiotes* (*Aagayathamarai*)

Date: 25.8.2017

Place: Chennai

25/8/2017

PG Department of Gunapadam

C.L.BAID METHA COLLEGE OF PHARMACY

(An ISO 9001-2000 certified institute)

Jyothi Nagar, Old Mahabalipuram Road

Thoraipakkam, Chennai – 600 097

CERTIFICATE

This is to certify that the project entitled, **Toxicological and Pharmacological study on BHRAMAASTHIRAM & RASA CHENDHOORAM** in rats submitted in partial fulfilment for the degree of **M.D. (Siddha)** was carried out at C.L. BaidMetha college of Pharmacy, Chennai-97, in the Department of Pharmacology during the academic year of 2015-2016. It has been approved by the

IAEC No: IAEC/XLIV/20/CLBMCP/2016

C.L. Baid Metha College of Pharmacy CHENNAI-97 Thoraipakkam

Dr.P.Muralidharan

IAEC Secretary

The Tamil Nadu Dr.M.G.R.Medical University

69, Anna Salai, Guindy, Chennai - 600 032.

This Certificate is awarded to Dr/Mr/Mrs.....S. SEMALATHA.....

For participating as ~~Resource~~ ~~Person~~ / Delegate in the Twentieth Workshop on

"RESEARCH METHODOLOGY & BIOSTATISTICS"

*For **AYUSH Post Graduates & Researchers***

Organized by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University From 07th to 11th March 2016.

Dr.N.KABILAN, M.D.(S)
PROF & HEAD
DEPT.OF SIDDHA

Prof.**Dr.P.ARUMUGAM,** M.D.,
REGISTRAR i/c

Prof. **Dr.S.GEETHALAKSHMI,** M.D., Ph.D.,
VICE CHANCELLOR